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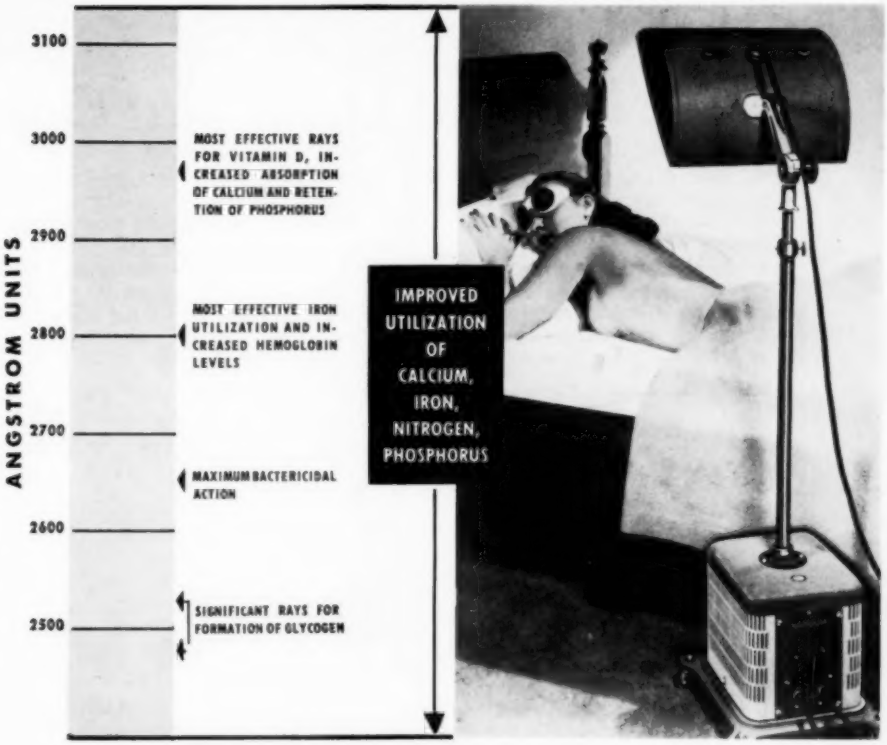

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Composition of 100 Gm., Edible Portion

	Kitchen-Cooked Beef Round[2]	Canned Roast Beef[2]
Water	59%	60%
Calories	233	224
Protein	27 Gm.	25 Gm.
Fat	13 Gm.	13 Gm.
Minerals, total	1.3 Gm.	2 Gm.
Phosphorus	224 mg.	116 mg.
Iron	3.4 mg.	2.4 mg.
Thiamine	0.08 mg.	0.02 mg.
Riboflavin	0.22 mg.	0.23 mg.
Niacin	5.5 mg.	4.2 mg.

The canning procedure has little effect upon the biologic value of the protein of meat.[3] In a recent study of the amounts of 18 amino acids in processed meats, canned beef, pork and lamb were generally similar in amino acid composition to fresh and kitchen-cooked samples.[4] Canned meat also is an excellent source of iron.

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REFERENCES

1. Howe, P. E.: Foods of Animal Origin, Handbook of Nutrition, American Medical Association, ed. 2, Philadelphia, The Blakiston Company, 1951, chap. 26, p. 637.
2. Watt, B. K., and Merrill, A. L.: Composition of Foods—Raw, Processed, Prepared, Agriculture Handbook No. 8, United States Department of Agriculture, 1950.
3. Stewart, J. A., and Clark, B. S.: The Canned Food Reference Manual, American Can Company Research Division, ed. 3, New York, American Can Company, 1947.
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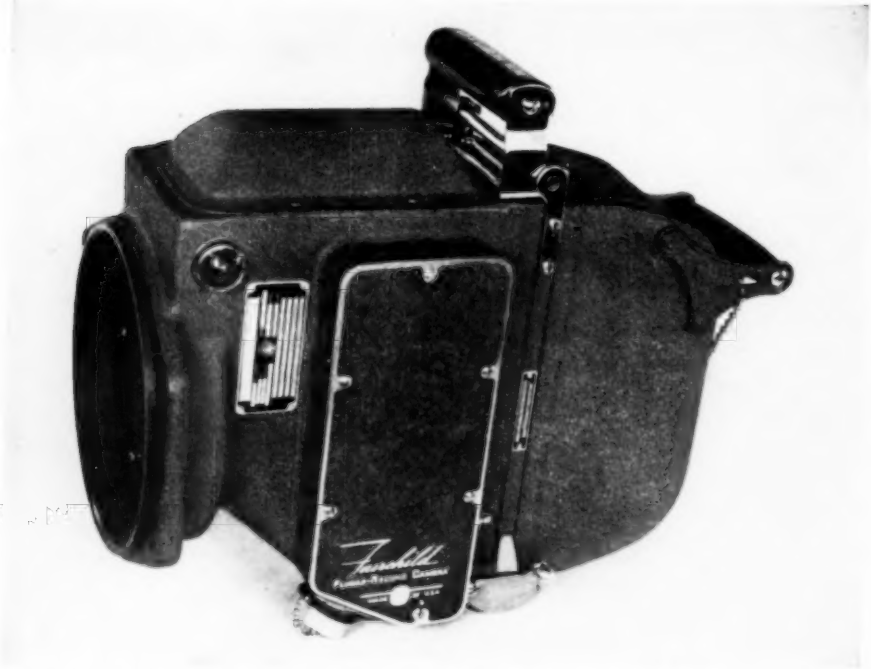

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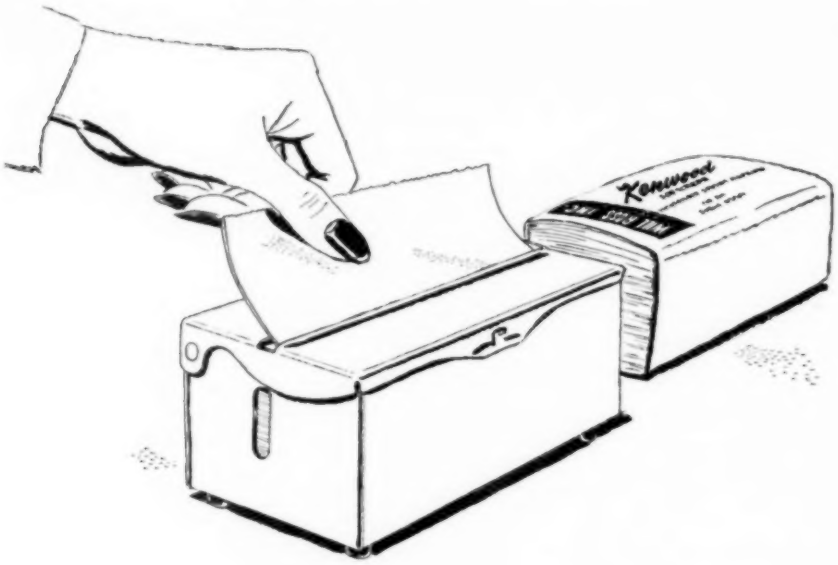

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THE AMERICAN REVIEW
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VOLUME 65 *April 1952* NUMBER 4

CHEMOTHERAPY OF EXPERIMENTAL TUBERCULOSIS[1]

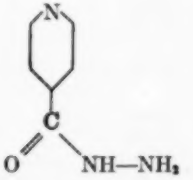
V. Isonicotinic Acid Hydrazide (Nydrazid[2]) and Related Compounds

JACK BERNSTEIN, WILLIAM A. LOTT, BERNARD A. STEINBERG, AND HARRY L. YALE

(Received for publication January 19, 1952)

INTRODUCTION

A class of compounds showing high activity against experimental tuberculosis in the mouse has been subjected to systematic study in this laboratory. One of the most active compounds in this series is isonicotinic acid hydrazide (Nydrazid).



In the present report, data are presented on isonicotinic acid hydrazide (Nydrazid) as well as on a number of related compounds, together with a brief discussion of the relationship of structure to activity. In mice infected with the Ravenel strain of *M. tuberculosis* var. *bovis*, isonicotinic acid hydrazide produced a significant increase in survival time when administered in a dose lower than any synthetic compound or natural product hitherto tested in this laboratory. Moreover, the ratio of the maximum acceptable level, when administered in powdered diet, to the minimum effective dose is extremely high. Following this lead a number of structurally related compounds were synthesized and evaluated for their antituberculous activity.

EXPERIMENTAL RESULTS

In table 1 are shown the comparative data on the antituberculous activity of isonicotinic acid hydrazide and *p*-aminosalicylic acid. Those compounds which showed significant delays in survival time are recorded in table 2.

[1] From the Squibb Institute for Medical Research, New Brunswick, New Jersey.
[2] Proprietary name, E. R. Squibb & Sons.

Of the compounds listed in the present report, only those which are derivatives of isonicotinic acid hydrazide were highly active. None of the aliphatic hydrazides showed any significant activity in the mouse. None of the simple aromatic hydrazides exhibited appreciable *in vivo* activity. In the pyridine monocarboxylic acid series, nicotinic acid hydrazide was inactive; picolinic acid hydrazide showed activity at one-half, but not one-quarter, of its maximum acceptable level. The presence of the hydrazide group is necessary for activity in the mouse, for the corresponding amide and hydroxamic acid were inactive. Data

TABLE 1

COMPARATIVE ANTITUBERCULOUS ACTIVITY IN THE MOUSE OF ISONICOTINIC ACID HYDRAZIDE (NYDRAZID) AND *p*-AMINOSALICYLIC ACID (PAS)

COMPOUND	LEVEL IN DIET	MEASURED DRUG INTAKE	INCREASE IN T_{50}	TOTAL OF SURVIVING MICE
	per cent	*mg/kg*		
Isonicotinic acid hydrazide	0.016	25	>15	10/10
Isonicotinic acid hydrazide	0.008	15	>15	10/10
PAS	0.75	1,300	5.5	0/10
PAS	0.375	650	3.5	0/10
Isonicotinic acid hydrazide	0.016	25	>15	10/10
Isonicotinic acid hydrazide	0.008	13	>15	10/10
Isonicotinic acid hydrazide	0.004	7	>15	10/10
Isonicotinic acid hydrazide	0.002	3	8.5	1/10
PAS	0.75	1,100	6.5	0/10
PAS	0.375	650	4.0	0/10
Isonicotinic acid hydrazide	0.002	3	>15	7/10
Isonicotinic acid hydrazide	0.001	2	7.0	0/10
PAS	0.75	1,300	7.0	0/10
PAS	0.375	600	3.5	0/10
Isonicotinic acid hydrazide	0.002	4	>15	8/10
PAS	0.75	1,300	9.0	0/10
PAS	0.375	650	5.0	0/10

on other derivatives of isonicotinic acid hydrazide and related compounds will be reported in a subsequent paper in this series.

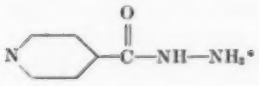
Those compounds inactive *in vivo* showed low activity *in vitro* in a standardized test (1). Compounds active *in vivo* showed high activity *in vitro* or could conceivably be broken down *in vivo* to isonicotinic acid hydrazide.

The compounds were tested in a standardized *in vivo* test against *M. tuberculosis* var. *bovis*, strain Ravenel (2).

In this test, groups of ten mice of the CF #1 strain were infected intravenously. The test drug was administered by mixing it with finely ground Rockland mouse pellets and letting the mice eat *ad libitum* for 23 days (21 days post infection). The criteria used for detection of activity against tuberculosis were: (*1*) the increase in T_{50} (time of death of 50 per cent of the mice) between the test mice and the untreated controls; and (*2*) for highly active

TABLE 2

COMPOUNDS ACTIVE AGAINST TUBERCULOSIS IN THE MOUSE

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG *in vitro*	MAXIMAL ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}
	γ/ml	mg/kg	mg/kg	days
	0.02	64	25	15+
			25	15+
			15	15+
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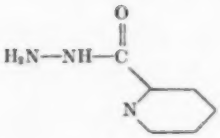
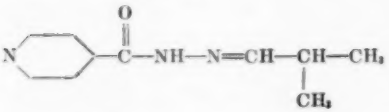
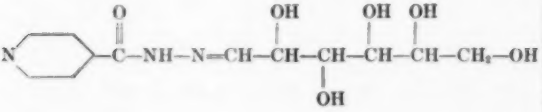
			7	15+
			4	15+
			3	15+
			3	8.5†
			2	7.0†
	0.15	125	40	15+
			25	1.5
	0.1	64	30	15+
			15	15+
			6	15+
			3	3
	0.15	1,000	400	15+
			250	15+
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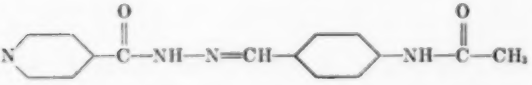
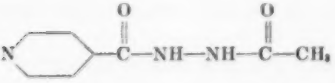
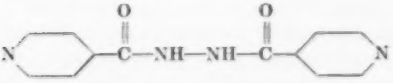
			25	15+
	0.08	>2,000	900	15+
			450	15+
			250	8
			200	2
			25	0
	15	1,000	550	15+
			250	12.5
	8.0	500	200	15+
			100	15+
			50	3
			15	1.5

* Isonicotinic acid hydrazide (Nydrazid).

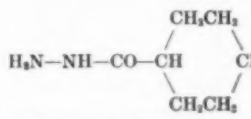
† An increase in T_{50} of three days or more is considered significant, $P < 0.01$.

compounds, the number of mice which survived the test period (35 days). In this standardized infection, the T_{50} of the untreated controls is usually between 17.5 and 20 days. No control mice survived for 25 days. Mice fed 0.375 per cent and 0.75 per cent of *p*-aminosalicylic acid (PAS) served as drug controls. *p*-Aminosalicyclic acid at the levels given produced approximately a four- and seven-day increase in T_{50}, respectively.

Data on the other compounds are listed in tables 2 to 5 inclusive. In table 3 the aliphatic and alicyclic compounds are listed. In table 4, the results of tests done with the aromatic derivatives are presented, and the inactive heteroaromatic compounds related to isonicotinic acid hydrazide are listed in table 5.

TABLE 3

ALIPHATIC AND ALICYCLIC HYDRAZIDES

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG *in vitro*	MAXIMAL ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}
	γ/ml	mg/kg	mg/kg	days
$CH_3—CO—NH—NH_2$	150	64	30	0.0
			15	−1.5
$CH_3—CO—NH—NH—CO—CH_3$	600	32	15	0.5
			7	−2.0
$H_2N—NH—CO—CH=CH—CO—NH—NH_2$	80	500	200	−1.0
			120	0.5
$H_2N—NH—CO—CH_2CH_2—CO—NH—NH_2$	25	250	110	1.5
			50	−0.5
$CH_3—(CH_2)_{16}—CO—NH—NH_2$	3	125	55	1.5
			25	0.5
 (Trans)	300	1,000	400	0.5
			230	0.0

In each table are recorded the structure of the compound, the approximate maximum acceptable level of the drug, the measured drug intake averaged over the first seven days in the therapeutic test, the corresponding change in T_{50}, and the minimal inhibitory concentration *in vitro* for the BCG strain of *M. tuberculosis*.

The minimum effective daily dose of isonicotinic acid hydrazide when administered in the diet to the infected mouse was less than 2 mg. per kg. but may be close to this figure in view of the high slope of the dose response curves for related compounds. The maximum acceptable level of isonicotinic acid hydrazide in the diet corresponds to a daily drug intake of 64 mg. per kg. When mice were given daily subcutaneous injections of the drug for seven days, the corresponding maximum tolerated dose was found to be 125 mg. per kg. The close correlation of these two figures suggests that the material was well absorbed from the ali-

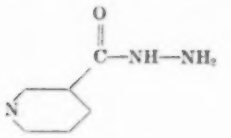
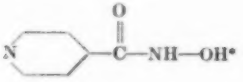
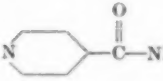
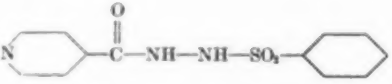
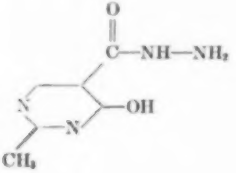
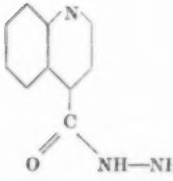
TABLE 4

AROMATIC HYDRAZIDES AND RELATED COMPOUNDS

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG *in vitro*	MAXIMAL ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}
	γ/ml	mg/kg	mg/kg	days
C_6H_5—CO—NH—NH_2	0.6	64	25	1.0
			14	1.5
4—Cl—C_6H_4—CO—NH—NH_2	3.0	125	50	2.0
			30	0.5
4—Br—C_6H_4—CO—NH—NH_2	12	64	30	0.5
			14	−0.5
4—I—C_6H_4—CO—NH—NH_2	6.0	125	45	−3.5
			25	0.0
4—NO_2—C_6H_4—CO—NH—NH_2	12	250	100	−0.5
			60	0.0
2—NO_2—C_6H_4—CO—NH—NH_2	300	1,000	400	−2.0
			200	−1.0
3—NO_2—C_6H_4—CO—NH—NH_2	0.6	125	50	1.0
			25	0.0
4—NH_2—C_6H_4—CO—NH—NH_2	6.0	500	175	3.5
			100	1.5
2—NH_2—C_6H_4—CO—NH—NH_2	30	250	100	2.0
			50	1.5
3—NH_2—C_6H_4—CO—NH—NH_2	3.0	250	100	1.0
			50	−0.5
4—$(CH_3)_2N$—C_6H_4—CO—NH—NH_2	6.0	250	100	0.0
			70	0.5
4—OH—C_6H_4—CO—NH—NH_2	12	1,000	500	2.5
			250	1.0
4—(H_3C—O)—C_6H_4—CO—NH—NH_2	8.0	125	50	0.0
			25	0.5
3—(H_3C—O)—4—OH—C_6H_3—CO—NH—NH_2	30	>2,000	750	−3.0
			350	−1.0
4—(H_2N—NH—CO)—C_6H_4—CO—NH—NH_2	3.0	2,000	700	−0.5
			400	−1.5
4—Cl—C_6H_4—CH_2—CO—NH—NH_2	10	1,000	400	−1.0
			200	1.0
C_6H_5—CH_2—CO—NH—NH_2	12	250	100	0.5
			55	0.5
C_6H_5—CH=CH—CO—NH—NH_2	0.4	>2,000	1,900	−1.5
			900	1.5
$(C_6H_5)_2$CH—CO—NH—NH_2	100	2,000	900	−0.5
			450	−0.5
$(C_6H_5)_2$C(OH)—CO—NH—NH_2	100	500	200	0.0
			100	2.5
C_6H_5—CH(OH)—CO—NH—NH_2	40	125	50	−3.0
			30	−3.0
4—NH_2—C_6H_4—SO_2—NH—NH_2	25	64	25	−1.5
			13	0.0
4—NO_2—C_6H_4—NH—NH—CO—CO—O—C_2H_5	0.03	64	25	−2.0
			13	1.0

TABLE 5

INACTIVE HETEROAROMATIC COMPOUNDS RELATED TO ISONICOTINIC ACID HYDRAZIDE

STRUCTURE	MINIMUM INHIBITING CONCENTRATION FOR BCG *in vitro*	MAXIMAL ACCEPTABLE DRUG INTAKE DAILY	MEASURED DRUG INTAKE DAILY	CHANGE IN T_{50}
	γ/ml	mg/kg	mg/kg	days
	2.0	250	100 50	1.0 0.5
	120	1,000	400 200	1.0 0.5
	1,000	2,000	1,400 900	−2.0 −0.5
	3.0	125	60 30	1.5 1.0
	400	>2,000	1,000 500	0.0 1.0
	80	500	200 100	0.0 0.0

* Gardner, T. S., Winis, E., Smith, F. A.: The synthesis of compounds for the chemotherapy of tuberculosis: II. Hydroxamic acid derivatives, J. Am. Chem. Soc., 1951, *73*, 5455. Compound reported to be inactive.

mentary canal of the mouse. Thus, the behavior of this compound is in contrast to that of the thiosemicarbazones which are poorly absorbed at high levels in the mouse, rat, and dog (3).

Because of the high activity *in vivo*, isonicotinic acid hydrazide was tested, using a delayed therapy technique. The data from these experiments are presented in table 6. The level of isonicotinic acid hydrazide was one-quarter of the maximum acceptable level and was known to be effective. It is evident that after seven days' delay in treatment all mice survived when treated in diet or subcutaneously. When treatment was delayed fourteen days, most of the mice were

TABLE 6

DELAYED THERAPY TEST—ISONICOTINIC ACID HYDRAZIDE (NYDRAZID)

COMPOUND	DAILY DOSE	ROUTE OF TREATMENT	TREATMENT STARTED	INCREASE IN T_{50}	TOTAL SURVIVING MICE
	mg/kg			*days*	
Isonicotinic acid hydrazide	15	diet	2 days before infection	>15	10/10
PAS	1,200	diet	2 days before infection	6.0	0/10
Isonicotinic acid hydrazide	9	diet	7 days after infection	>15	10/10
PAS	800	diet	7 days after infection	5.0	0/10
Isonicotinic acid hydrazide	12	diet	14 days after infection	—	4/8*
PAS	600	diet	14 days after infection	—	0/10
Isonicotinic acid hydrazide	16	subcutaneous	2 hours after infection	>15	10/10
Streptomycin HCl	13	subcutaneous	2 hours after infection	4.5	1/9
Isonicotinic acid hydrazide	16	subcutaneous	7 days after infection	>15	10/10
Streptomycin HCl	13	subcutaneous	7 days after infection	1.0	0/10
Isonicotinic acid hydrazide	16	subcutaneous	14 days after infection	—	7/9
Streptomycin HCl	12	subcutaneous	14 days after infection	—	0/10

* Two of the 10 mice died before fourteenth day, i.e., before start of drug treatment.

moribund. Despite this advanced stage of the disease, subcutaneous administration resulted in a high percentage of complete recovery.

SUMMARY

A class of compounds showing high antituberculous activity is reported for the first time. Of these, the most active is isonicotinic acid hydrazide (Nydrazid). In a standardized infection of mice with the Ravenel strain of *M. tuberculosis*, the minimal effective dose of this compound is 1/700 that of *p*-aminosalicylic acid.

SUMARIO

Quimioterapia de la Tuberculosis Experimental. V. La Hidracida del Acido Isonicotínico (Nydrazid) y los Compuestos Afines

Esta es la primera vez que se describe un grupo de compuestos dotados de elevada actividad antituberculosa. De ellos, el más activo es la hidracida del ácido isonicotínico (Nydrazid). En una infección uniformizada de los ratones con la cepa Ravenel del *M. tuberculosis*, la mínima dosis eficaz de dicho compuesto representa 1/700 de la del ácido *p*-aminosalicílico.

RESUME

Chimiothérapie de la tuberculose expérimentale: V. l'hydrazide de l'acide isonicotinique (Nydrazid) et composés apparentés

Une classe de composés montrant une activité antituberculeuse élevée a fait, pour la première fois, l'objet d'un rapport. Parmi ceux-ci, le plus actif est l'hydrazide de l'acide isonicotinique (Nydrazid). Dans l'infection standard de la souris par la souche Ravenel du *M. tuberculosis*, la dose efficace minima de ce composé est 1/700e de la valeur de celle de l'acide para-aminosalicylique.

REFERENCES

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(2) Rake, G., Jambor, W., McKee, C. M., Pansy, F., Wiselogle, F. Y., and Donovick, R.: The use of the mouse in a standardized test for antituberculous activity of compounds of natural or synthetic origin: III. The standardized test, Am. Rev. Tuberc., 1949, *60*, 121.

(3) Burke, J. C., and Titus, E. O.: The pharmacology of *p*-formylacetanilide thiosemicarbazone (Myvizone) in animals, Nat. Tuberc. A. Tr., 1950, p. 118.

ANTITUBERCULOUS PROPERTIES OF HYDRAZINES OF ISONICOTINIC ACID (RIMIFON,[1] MARSILID[2])[3,4]

WILLIAM STEENKEN, JR., AND EMANUEL WOLINSKY

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In this report are presented preliminary observations concerning the effect of two hydrazines of isonicotinic acid (isonicotinic acid hydrazide [Rimifon] and 1-isonicotinyl-2-isopropyl hydrazine [Marsilid]) on the tubercle bacillus *in vitro* and on tuberculosis of the experimental animal (6). Data are presented on the tuberculostatic action of the drugs against the human virulent strain of *M. tuberculosis* (H37Rv) in several different types of media; the *in vitro* tuberculocidal action against the same strain; the attempted development of drug resistance *in vitro*; the effect of the drugs in tuberculosis of guinea pigs infected subcutaneously and intracerebrally, and of rabbits infected intravenously with virulent bovine tubercle bacilli (strain No. 4228-4); and the toxicity of the drugs for rabbits and guinea pigs.

OBSERVATIONS

In Vitro Activity

The inhibitory effect of the drugs on the growth of the H37Rv strain of *M. tuberculosis* in three different types of liquid media and in a solid medium is illustrated in table 1.

One of the drugs, 1-isonicotinyl-2-isopropyl hydrazine dihydrochloride, (Marsilid), produced complete inhibition of growth for a period of fourteen days in concentrations of 1.6 to 3.1 γ per ml. in media without serum. The presence of 10 per cent horse serum increased the inhibitory concentration approximately fourfold. Isonicotinic acid hydrazide (Rimifon) exhibited greater *in vitro* activity, requiring only 0.025 to 0.05 γ per ml. for complete inhibition of growth for fourteen days. In the synthetic medium with serum, approximately twice the concentration of drug was necessary for growth inhibition as in the plain synthetic medium.

The tuberculocidal effect of one of these drugs is indicated in table 2, which presents the results of a preliminary experiment.

An inoculum of H37Rv, contained in 0.5 ml. of a seven-day culture in Tween-albumin medium, was added to each of 3 flasks of the same medium, 2 of which contained 10 and 1.0 γ of 1-isonicotinyl-2-isopropyl hydrazine per ml., respectively; the third inoculated flask was a control which contained no drug. Plate

[1, 2] Proprietary names (Hoffman-LaRoche) for isonicotinic acid hydrazide and 1-isonicotinyl-2-isopropyl hydrazine, respectively.

[3] From the Trudeau Laboratory of the Trudeau Foundation for the Clinical and Experimental Study of Pulmonary Disease, Trudeau, New York.

[4] This study was aided (in part) by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service, and Hoffman-LaRoche, Inc.

counts were made on oleic-acid albumin agar according to the drop method described by Fenner (1) at the intervals shown in the table. It may be seen that 10 γ per ml. of the hydrazine (Marsilid) produced a fairly rapid decrease in the number of viable organisms. The killing effect of various concentrations of these drugs under many different conditions is being investigated in greater detail, and the results will be reported at a later date.

An attempt is being made to produce strains of H37Rv resistant to these drugs by serial transfers in drug-containing Tween-albumin medium. So far,

TABLE 1

TUBERCULOSTATIC EFFECT IN VITRO OF HYDRAZINES OF ISONICOTINIC ACID (RIMIFON, MARSILID)

Inoculum: Seven-Day Culture of H37Rv in Tween-Albumin Medium, 0.1 cc. for Tubes of Liquid Medium, One Double Loopful for Plates of Solid Medium. *Incubation:* 37°C., Fourteen Days for Liquid Media, Twenty-One Days for Solid Medium

DRUG	MINIMAL INHIBITORY CONCENTRATION IN γ PER ML.			
	Tween-Albumin Liquid Medium	Proskauer and Beck Synthetic Medium	Proskauer and Beck with 10 Per Cent Horse Serum	Oleic-Acid Albumin Agar
1-isonicotinyl-2-isopropyl hydrazine dihydrochloride	3.1	1.6	6.2	2.5
Isonicotinic acid hydrazide	0.05	0.025	0.05	0.25

TABLE 2

TUBERCULOCIDAL EFFECT OF 1-ISONICOTINYL-2-ISOPROPYL HYDRAZINE (MARSILID)

Flasks of Tween-Albumin Liquid Medium Incubated at 37°C. Figures Indicate Number of Viable Units per Ml. of Medium

CONCENTRATION OF DRUG	IMMEDIATELY AFTER INNOCULATION	24 HOURS	5 DAYS
0	200,000	250,000	2,500,000
1.0 γ per ml.	200,000	260,000	1,000,000
10 γ per ml.	200,000	15,000	<10

a fivefold increase in resistance to 1-isonicotinyl-2-isopropyl hydrazine has been noted after seven transfers at two-week intervals.

Toxicity for Experimental Animals (5)

The results of trials designed to show the maximum tolerated dosage for long-term administration yielded the following data on the chronic toxicity of the two drugs for guinea pigs and rabbits:

Guinea pigs, parenteral administration: 1-Isonicotinyl-2-isopropyl hydrazine (Marsilid) was administered intramuscularly to guinea pigs. The original sample of this drug was the dihydrochloride salt which gave highly acid aqueous solutions and, when injected intramuscularly, these solutions produced local reactions consisting of necrosis and induration. When they were neutralized, how-

ever, or when the drug in the form of the free base was used, the local reactions were minimal. A dosage of 20 mg. twice daily (70 mg. per kg. per day) for eight days produced steady weight loss, and 20 mg. once daily (35 mg. per kg. per day) produced loss of weight and an occasional death after three weeks. A dosage regimen of 10 mg. twice daily (approximately 35 mg. per kg. per day) was tolerated well for sixty days.

Isonicotinic acid hydrazide (Rimifon) in dosage up to 35 mg. per kg. per day was tolerated well for six weeks.

Guinea pigs, oral administration: 1-Isonicotinyl-2-isopropyl hydrazine was given to 2 guinea pigs in a dosage of 35 mg. twice daily (90 mg. per kg. per day). One animal died on the fifth day, with blood and many casts in the urine. The other guinea pig lost weight for thirteen days. Two other guinea pigs received 35 mg. once daily (50 mg. per kg. per day). One of these animals died on the tenth day. The lower ileum contained bloody material, and gross blood and casts were present in the urine. The other animal lost weight for thirteen days. In 4 animals 25 mg. once daily (40 mg. per kg. per day) were tolerated well for two weeks.

Isonicotinic acid hydrazide (Rimifon) up to 40 mg. per kg. per day was well tolerated when administered by the oral route for a two-week period.

Rabbits, parenteral administration: The intramuscular administration of 1-isonicotinyl-2-isopropyl hydrazine in a dosage of 100 mg. twice daily (60 mg. per kg. per day) resulted in the death of one rabbit on the fourth day. In other rabbits a dosage of 60 mg. twice daily intramuscularly (45 mg. per kg. per day) for five days produced loss of appetite and weight loss in 3 animals, and 30 mg. twice daily intramuscularly (20 mg. per kg. per day) were tolerated well for seventy days.

Isonicotinic acid hydrazide in a dosage of 15 mg. twice daily intramuscularly (9 mg. per kg. per day) was tolerated well for twenty-one days.

In Vivo Activity in Guinea Pigs Infected Subcutaneously

Twenty guinea pigs, weighing between 500 and 900 gm., were infected subcutaneously in the left inguinal region with 0.1 mg. (dry weight) of the H37Rv strain of human tubercle bacilli. All showed a markedly positive skin reaction to 5 per cent Old Tuberculin twenty days later. On the twenty-first day, 10 of these animals were started on treatment with 1-isonicotinyl-2-isopropyl hydrazine (Marsilid). The initial dosage was 20 mg. of the dihydrochloride salt intramuscularly once daily, but after twenty-six days of treatment it became apparent that the animals were not able to tolerate this amount. As noted previously, solutions of the dihydrochloride salt produced large areas of necrosis and induration at the sites of injection. After a four-day rest period, therapy was continued in the form of capsules given by mouth and containing 35 mg. of the dihydrochloride. One capsule was given once daily for ten days, after which the dosage was again reduced to 25 mg. orally once daily.

Thirty days after treatment was begun, the tuberculin skin reactions were again tested. All of the control animals still exhibited markedly positive reac-

tions with necrosis, while of the 7 treated animals still alive 5 showed only pale atypical reactions, and 2 were negative.

Seventy-three days after infection, all surviving animals were killed. The amount of gross tuberculous disease found in the organs of these animals is indicated in table 3.

TABLE 3

RESULTS OF TREATMENT WITH 1-ISONICOTINYL-2-ISOPROPYL HYDRAZINE (MARSILID) IN EXPERIMENTAL TUBERCULOSIS OF GUINEA PIGS INFECTED SUBCUTANEOUSLY

Therapy Begun Twenty-One Days After Infection. Survivors Killed Seventy-three Days After Infection

Guinea Pig Number	Survival in Days	Days of Treatment	Index of Tuberculosis*				
			Lungs	Liver	Spleen	Lymph Nodes	Totals
1	73 K†	0	1.5	3	2.5	3	10.0
2	73 K	0	2.5	3	3	3.5	12.0
3	73 K	0	1.5	2.5	2.5	3	9.5
4	73 K	0	2.5	2.5	2.5	3	10.5
5	73 K	0	2.5	3	3	3	11.5
6	73 K	0	1.5	2.5	2.5	3.5	10.0
7	73 K	0	3	3.5	3.5	3.5	13.5
8	73 K	0	2.5	2.5	2.5	3	10.5
9	73 K	0	2	2.5	3	3	10.5
10	73 K	0	2.5	2.5	2.5	3	10.5
Averages			2.2	2.75	2.75	3.15	10.85
1	34 D†	13	1	0	±	1.5	3.0
2	42 D	21	0	0	0	1	1.0
3	47 D	26	0	0	0	1	1.0
4	62 D	41	0	0	0	0	0
5	70 D	49	0	0	0	0	0
6	72 D	51	0	0	0	1	1.0
7	73 K	52	0	0	0	±	0.5
8	73 K	52	0	0	0	±	0.5
9	73 K	52	0	0	0	1	1.0
10	73 K	52	0	0	±	1	1.5
Averages‡			0	0	0.05	0.67	0.72

* Each organ (lungs, liver, spleen, lymph nodes) was assigned a value of 0 to 4 according to the amount of gross tuberculous disease. Maximum total for each animal was therefore 16.

† K, killed; D, died.

‡ Excluding animal which died after only thirteen days of treatment.

It will be noted that 6 of the treated animals did not survive until the end of the experiment, but died apparently of drug toxicity after thirteen, twenty-one, twenty-six, forty-one, forty-nine, and fifty-one days of treatment. Obviously, the initial dosage was too large, but the animals that died after only three or

four weeks of treatment did not show appreciable differences in gross tuberculous disease from those that survived the full course of fifty-two days.

Macroscopic examination: The untreated control animals exhibited the usual widespread progressive lesions in the lungs, liver, spleen, and lymph nodes. The treated animals, on the other hand, showed very little macroscopic evidence of tuberculous infection. None had gross lesions in the lungs or the liver (aside from the one animal which died after only thirteen days of treatment), and only 2 had what were interpreted as questionable lesions in the spleen. Most of the treated animals showed slight enlargement of, and a small amount of caseous material in, the local inguinal and iliac lymph nodes as the only remaining indication of tuberculous infection.

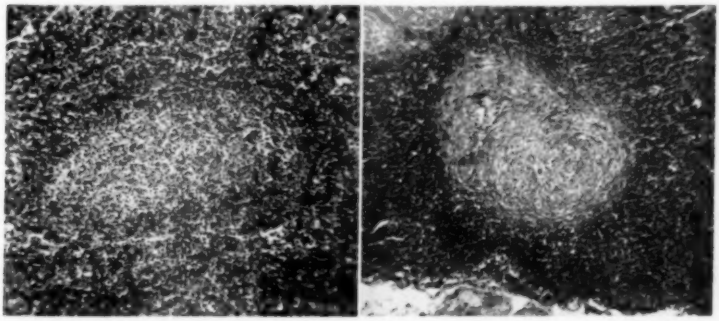

FIG. 1. Microscopic sections from tuberculous guinea pigs treated with 1-isonicotinyl-2-isopropyl hydrazine (Marsilid). (100×)

Left: Spleen of animal which died after forty-one days of treatment. A tuberculous lesion replaces all but the peripheral margin of a Malpighian body. The lesion consists mainly of epithelioid cells and shows pronounced lymphocytic infiltration, suggesting regression. Acid-fast bacilli found in the lesion in small numbers.

Right: Hepatic lymph node from animal killed after fifty-two days of treatment. The lesion is a classic epithelioid cell tubercle with a well-defined peripheral fibrous tissue wall. Acid-fast bacilli found in small numbers.

Macerated splenic tissue and caseous material from the local lymph nodes of the 4 animals killed after fifty-two days of treatment were subinoculated into normal guinea pigs in an attempt torecover viable tubercle bacilli. After nine weeks, intracutaneous tuberculin reactions in the subinoculated animals indicate that at least 3 of the originally treated guinea pigs still harbored virulent organisms.

Microscopic examination: Microscopic examination of the organs of 6 treated guinea pigs revealed the following findings: None of the lung sections contained tuberculous lesions. Only one of the 6 livers showed incomplete central necrosis; none were the site of tuberculous changes. Epithelioid tubercles with peripheral fibrous tissue walls were seen in the hepatic lymph nodes of 5 animals. Small numbers of acid-fast bacilli were demonstrated in these lesions.

The spleens from 4 animals showed no tuberculous lesions. One had an occasional small epithelioid tubercle in a Malpighian body (figure 1), and another exhibited a few small epithelioid tubercles and hyperplasia of the Malpighian bodies. The kidneys of one animal (which had died after forty-one days of treatment) contained many tubular casts. No other abnormal findings were noted.

In figure 1 are shown representative microscopic sections from the organs of the treated animals.

Guinea Pigs Infected Intracerebrally

Fifteen guinea pigs were infected intracerebrally with the virulent H37Rv microorganisms in the manner previously described (2). Five animals remained untreated; 5 were treated with amithiozone[5], and 5 were treated with 1-isonicotinyl-2-isopropyl hydrazine, starting the day after infection. In table 4 the dosage of the drugs used and the results of treatment are given.

TABLE 4

EFFECT OF 1-ISONICOTINYL-2-ISOPROPYL HYDRAZINE (MARSILID) ON EXPERIMENTAL TUBERCULOUS MENINGITIS IN GUINEA PIGS INFECTED INTRACEREBRALLY

Treatment Started Day After Infection

TREATMENT	SURVIVAL TIME, DAYS AFTER INFECTION
None (Controls)	17, 20, 21, 24, 25 Mean: 21
Amithiozone by mouth: 45 mg. 1 × day for 11 days 45 mg. 2 × day for 14 days No drug for 3 days 45 mg. 1 × day thereafter until death	26, 32, 33, 37, 40 Mean: 33
1-Isonicotinyl-2-isopropyl hydrazine, intramuscularly: 10 mg. 1 × day for 11 days 10 mg. 2 × day for 36 days 20 mg. 1 × day for 13 days	All appeared normal during 60 days of treatment.

[5] 4-Acetylaminobenzaldehyde thiosemicarbazone (Tibione), Schenley Laboratories, Inc.

FIG. 2. Representative chest roentgenograms of control and hydrazine-treated rabbits after intravenous infection with virulent bovine tubercle bacilli.

Upper Row: Control Rabbit

Left: preinfection, no abnormal shadows

Middle: fourteen days after infection, no abnormal shadows

Right: twenty-three days after infection, advanced miliary lesions throughout both lungs

Lower Row: Rabbit, treatment with 1-isonicotinyl-2-isopropyl hydrazine (Marsilid) begun day after infection

Left: preinfection, no abnormal shadows

Middle: twenty-eight days after infection, no abnormal shadows

Right: seventy-one days, no abnormal shadows

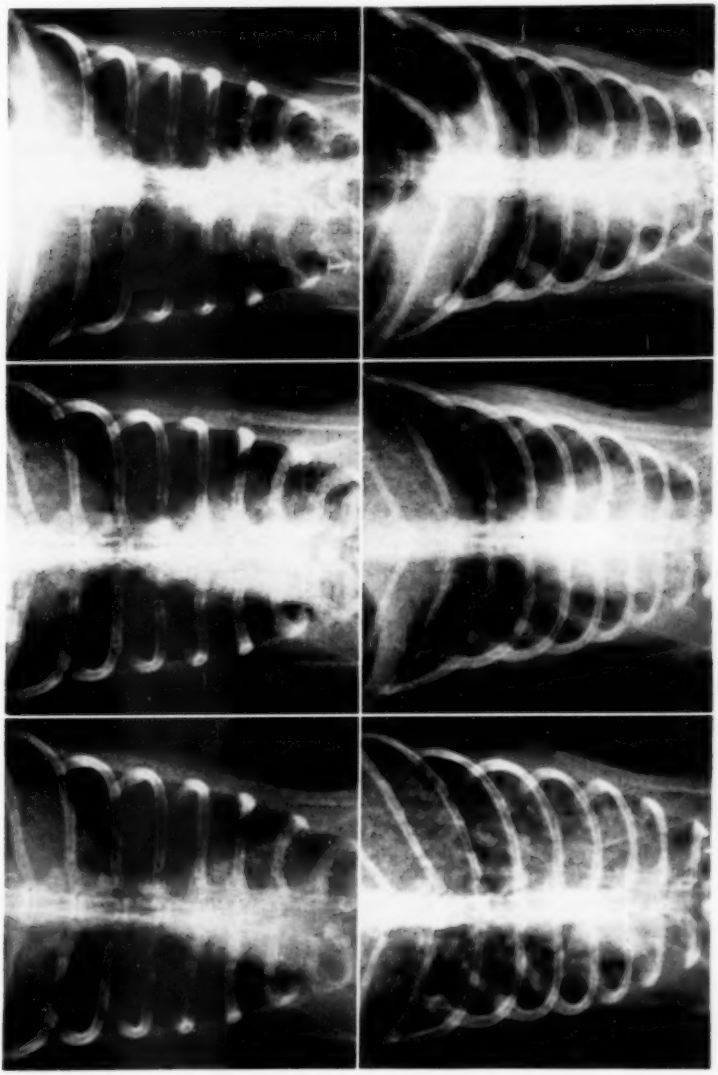

FIG. 2

Previous experience with the intracerebral method of infection (2) has demonstrated that it is rapidly and uniformly fatal to untreated animals. In the present experiment, all of the controls died within twenty-five days after infection. The survival time of those treated with amithiozone was increased significantly, but all had died by the fortieth day.

The animals treated with the hydrazine were still alive and apparently well after sixty days, at which time treatment was terminated. Skin tests with 5 per cent Old Tuberculin on the forty-sixth day of treatment were negative in all of the 5 guinea pigs. Twenty-two days after therapy was discontinued, 4 animals were still negative and one showed a doubtful reaction to tuberculin.

The fate of these 5 guinea pigs after cessation of treatment will be reported subsequently.[6] It will be recalled that animals infected in a similar manner and treated with streptomycin for one hundred and forty-seven days relapsed and died within forty-three days (2).

Rabbits Infected Intravenously

Rabbits weighing about 3.5 kg. were infected intravenously in the marginal ear vein with 0.01 mg. (dry weight) of virulent bovine tubercle bacilli (strain No. 4228–4). Previous experience with this culture indicated that such dosage was sufficient to kill all rabbits within three to six weeks. Five rabbits were held as untreated controls, and 3 were given 1-isonicotinyl-2-isopropyl hydrazine, (Marsilid) starting the following day. The drug was administered in dosage of 30 mg. twice daily, intramuscularly, for seventy-one days. The progress of the miliary lesions was followed by serial chest roentgenograms,[7] as described in a previous communication (3).

Miliary pulmonary lesions could be distinguished in the roentgenograms of all of the control rabbits three weeks after infection. The 5 control animals died twenty-two, twenty-six, twenty-six, twenty-seven, and thirty days after infection. In the 3 treated animals, on the other hand, no lesions were visible on the roentgenograms and the animals remained well during the seventy-one days of treatment. The chest roentgenograms of one of the control and one of the treated rabbits, which are representative of both groups of animals, are presented in figure 2.

The subsequent course of the 3 animals taken off treatment after seventy-one days[8], as well as the results of treatment with this drug and with isonicotinic acid hydrazide on already established miliary pulmonary lesions in rabbits, will be presented in a future publication.

[6] Since this manuscript was submitted, 4 of these guinea pigs have died (thirty, thirty-eight, forty-one, and forty-two days after termination of treatment) and the fifth is showing progressive paralysis of the hindquarters. Macroscopic evidence of tuberculosis in those which died was limited to small lesions in the cervical lymph nodes in which acid-fast bacilli could be demonstrated by smear. The brains were removed intact and fixed in formalin for sectioning.

[7] The roentgenographic equipment was generously donated by The Lillia Babbitt Hyde Foundation.

[8] Pulmonary lesions became evident in all within ten weeks.

DISCUSSION

Both of these hydrazines are active *in vitro*, the parent compound (isonicotinic acid hydrazide) showing greater activity than the isopropyl derivative. Neither drug is more than slightly affected by the presence of serum in the medium. The fact that tubercle bacilli cannot readily be made highly resistant to these drugs by serial passage in Tween-albumin liquid medium does not necessarily mean that resistance will not appear in cultures from patients treated with the drugs for prolonged periods. Experience with para-aminosalicylic acid (PAS) has indicated that resistant organisms often may be recovered from patients under treatment with PAS alone, despite the fact that it is difficult to produce drug resistance by serial transfer of tubercle bacilli in drug-containing medium. The apparent tuberculocidal action of 1-isonicotinyl-2-isopropyl hydrazine in concentration of 10 γ per ml. is an encouraging characteristic and is being studied further.

In the experience of this laboratory, the dramatic beneficial effect of 1-isonicotinyl-2-isopropyl hydrazine on acute guinea pig tuberculosis surpasses that seen with any other single antibacterial agent except streptomycin. It has been observed (4) that tuberculous guinea pigs treated with streptomycin may exhibit a gradual decrease in skin sensitivity to tuberculin; but the change from highly positive necrotic skin tests to negative or doubtful reactions after one month of treatment, as observed in the experiment reported herein, has not been noted previously. That this alteration was not merely due to an effect of the drug on the reactivity of the skin was shown by the failure of the hydrazine, when administered for a three-day period to 2 guinea pigs with highly positive reactions, to cause a change in the cutaneous reaction to tuberculin. The failure of the skin to react to tuberculin was not the same as the phenomenon seen in tuberculous guinea pigs given cortisone, in which the skin reactions may lose their inflammatory aspect and become very pale, even though necrosis is evident.

1-Isonicotinyl-2-isopropyl hydrazine also proved to be highly effective in guinea pigs infected intracerebrally. Streptomycin and viomycin are the only other drugs of the many tested in this laboratory which afford equal protection against this type of infection. Guinea pigs treated with streptomycin for one hundred and forty-seven days, however, relapsed and died within twenty-two to forty-three days after the discontinuance of the drug (2). The course of the guinea pigs taken off treatment after sixty days in the present experiment will be followed with interest (*See* footnote 6). On the twenty-second day after treatment was stopped, 4 of the 5 animals showed negative skin reactions to Old Tuberculin, while one pig exhibited a doubtful reaction. It is therefore possible that any signs of brain damage which may develop will be the result of a hydrocephalus, or similar process, rather than of progressive tuberculosis (2).

In regard to the rabbit experiment, it should be emphasized that the culture used for infection of the animals was a highly virulent bovine strain capable of producing 100 per cent mortality in the dosage described. It must be concluded, therefore, that the hydrazine suppressed the miliary tuberculous infection for at

least seventy-two days[8]. The effect of the two drugs on established and advanced miliary disease, as well as on chronic cavitary disease in rabbits, is being investigated.

SUMMARY

The antituberculous activity of isonicotinic acid hydrazide (Rimifon) and its isopropyl derivative (Marsilid) has been investigated in the test tube and in experimental tuberculous infections of guinea pigs and rabbits. The preliminary observations herein reported indicate that these drugs have tuberculostatic and tuberculocidal action *in vitro* and produce a dramatic beneficial effect *in vivo* in the experimental animal.

Guinea pigs infected subcutaneously with virulent human tubercle bacilli and treated with 1-isonicotinyl-2-isopropyl hydrazine twenty-one days later revealed very little evidence of tuberculosis grossly when killed seventy-three days after infection. After thirty days of treatment, the tuberculin skin reactions, which had been highly positive at the beginning of therapy, were markedly altered.

Untreated guinea pigs infected intracerebrally with virulent human tubercle bacilli succumbed within twenty-five days, while those treated with 1-isonicotinyl-2-isopropyl hydrazine remained well during sixty days of treatment.

Rabbits treated with 1-isonicotinyl-2-isopropyl hydrazine for 71 days remained well and failed to develop miliary pulmonary lesions demonstrable by roentgenography after intravenous infection with highly virulent bovine tubercle bacilli, while control animals all died of advanced miliary tuberculosis within thirty days of infection. These rabbits, however, showed relapse of tuberculosis within ten weeks after treatment was stopped.

SUMARIO

Propiedades Antituberculosas de las Hidracinas del Acido Isonicotínico (Rimifón, Marsilid)

La actividad antituberculosa de la hidracida del ácido isonicotínico (Rimifón) y del derivado isopropílico del mismo (Marsilid) fué investigada en el tubo de ensayo y en las infecciones tuberculosas experimentales de los cobayos y los conejos. las observaciones preliminares aquí presentadas indican que dichas drogas ejercen acción tuberculoestática y tuberculocida *in vitro* y producen un efecto beneficioso teatral *in vivo* en el animal de experimentación.

Los cobayos infectados subcutáneamente con bacilos tuberculosos humanos virulentos y tratados con 1-isonicotil-isopropil hidracina veintiún días después revelaron muy pocos signos macroscópicos de tuberculosis al ser matados a los setenta y tres días de la infección. Al cabo de treinta días de tratamiento, estaban considerablemente modificadas las cutirreacciones a la tuberculina, que habían sido muy positivas al comenzar la terapéutica.

Los cobayos no tratados e infectados intracerebralmente con bacilos tuberculosos humanos virulentos sucumbieron en término de veinticinco días, en tanto que los tratados con 1-isonicotinil-isopropil hidracina permanecieron sanos durante sesenta días de tratamiento.

Los conejos tratados con 1-isonicotinil-isopropil hidracina permanecieron sanos y no manifestaron en los pulmones lesiones granúlicas observables con la radiografía después de la inyección endovenosa de bacilos tuberculosos bovinos virulentísimos, mientras que todos los animales testigos murieron de granulia en término de treinta días de la infección.

RESUME

Propriétés antituberculeuses des hydrazines de l'acide isonicotinique (Rimifon, Marsilid)

L'activité antituberculeuse de l'hydrazide de l'acide isonicotinique (Rimifon) et de son dérivé isopropyl (Marsilid) a été étudiée à la fois dans le tube à essai et dans l'infection tuberculeuse expérimentale du cobaye et du lapin. Les observations préliminaires rapportées ici indiquent que ces produits possèdent une action tuberculostatique et tuberculocide, *in vitro*, et qu'ils produisent des effets favorables spectaculaires *in vivo*, chez l'animal en expérience.

Chez les cobayes inoculés par voie sous-cutanée avec des bacilles tuberculeux humains virulents, et traités vint-et-un jours plus tard par 1-isonicotinyl-isopropyl hydrazine, l'examen macroscopique n'a révélé que peu de manifestations de tuberculose, quand ces animaux ont été tués soixante-treize jours après l'infection. Au bout de trente jours de traitement, les réactions cutanées à la tuberculine, qui s'étaient montrées fortement positives au commencement du traitement, étaient modifiées de façon marquée.

Les cobayes non traités, inoculés par voie intracérébrale avec des bacilles tuberculeux humains virulents, ont succombé dans les vingt-cinq jours, tandis que ceux traités avec 1-isonicotinyl-isopropyl hydrazine sont demeurés en bon état pendant les soixante jours du traitement.

Des lapins, traités avec 1-isonicotinyl-isopropyl hydrazine après inoculation avec des bacilles tuberculeux bovins très virulents, sont demeurés en bonne santé et il ne s'est pas produit de lésions pulmonaires miliares, décelables par la radiographie, tandis que les animaux témoins sont tous morts de tuberculose miliaire généralisée dans les trente jours qui ont succédé à l'inoculation.

Addendum

When incubation of the inoculated tubes of Tween-albumin medium containing isonicotinic acid hydrazide was continued for five weeks, growth eventually appeared in concentrations as high as 25 γ per ml. It has been demonstrated that this delayed growth consists of tubercle bacilli which have an increased resistance to the drug.

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PHARMACOLOGIC AND TOXICOLOGIC OBSERVATIONS ON HYDRAZINE DERIVATIVES OF ISONICOTINIC ACID (RIMIFON[1], MARSILID[2, 3])

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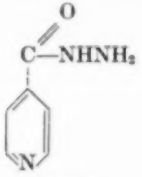
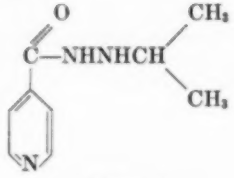
INTRODUCTION

The observations by Grunberg and Schnitzer (1), confirmed by Steenken and Wolinsky (2), of the marked chemotherapeutic activity against experimental tuberculosis of the hydrazine derivatives of isonicotinic acid synthesized by Fox (3) prompted additional toxicologic and pharmacologic investigations. The studies reported here were confined to two of these agents, i.e., isonicotinic acid hydrazide (Rimifon) and 1-isonicotinyl-2-isopropyl hydrazine (Marsilid). The formulas of these compounds are as follows:



Rimifon



Marsilid

These agents were studied in acute tests in mice, rats, guinea pigs, rabbits, cats, and dogs. The response of both anesthesized and unanesthetized animals, as well as that of isolated tissues, was observed and recorded in an endeavor to elicit the pharmacodynamic effects and the inherent toxicity of these agents.

In addition, the two compounds were administered daily over a period of thirteen weeks to two animal species (rats and dogs) in an effort to determine the probable limitations of dosage in man, with particular reference to hematologic and morphologic changes. These studies were carried out simultaneously with those made in the monkey, reported by Lewis and Zieper (4).

METHODS

GENERAL PHARMACOLOGY

Tests on isolated tissue included the evaluation of these agents for parasympathetic blocking activity on segments of rabbit intestine immersed in Locke's solution and for

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[1] Proprietary name, Hoffmann-La Roche, Inc., brand of Conilozene, formerly designated as Ro 2-3973.

[2] Proprietary name, Hoffmann-La Roche, Inc., brand of Coronozene, formerly designated as Ro 2-4572/1.

[3] From the Department of Pharmacology, Hoffmann-La Roche, Inc., Nutley, New Jersey.

antihistamine activity on segments of guinea pig intestine immersed in Tyrode's solution. Intestinal studies were also carried out in the dog with a Thiry-Vella fistula.

Tests for autonomic activity in unanesthetized animals also included a study of possible antisalivation effects in the rabbit. In this experiment, salivation was provoked by the subcutaneous administration of 15 mg. of pilocarpine per kg. A comparison was then made between the degree of salivary flow of control animals and those receiving, in addition, the hydrazine derivatives.

A second test for antisecretory activity consisted of observing the effect of the antituberculous agents on lacrimation in the rat, induced by the intraperitoneal injection of 10 mg. of methacholine per kg.

Observations for possible effects on pupillary size and local anesthesia were carried out by instillation of the hydrazine derivatives into the conjunctival sacs of the eyes of rabbits.

Tests for potential bronchiolytic activity were performed on guinea pigs in which bronchospasm was induced by an aerosol of either a 0.50 per cent solution of methacholine or a 0.33 per cent solution of histamine. The antituberculous agents were administered intraperitoneally thirty minutes prior to the aerosol exposure.

Tests for antipyretic activity were carried out in rabbits in which fever was induced by the intravenous administration of 0.1 cc. of a suspension of *E. coli* (six billion organisms per cc.). The hydrazides were administered orally one hour after the *E. coli*, and temperatures were recorded for a period of three hours.

In anesthetized animals[4] blood pressure effects of the intravenously administered drugs were manometrically measured after cannulation of the carotid artery. In dogs anesthetized with pentobarbital, the direct effect of increasing doses was observed on blood pressure as well as the indirect influence on the response to such procedures as stimulation of the vagus, occlusion of the carotid artery, and injections of acetylcholine and epinephrine.

The effect of the hydrazides on ganglionic transmission in the sympathetic nervous system was carried out in the cat (anesthetized with Dial-urethane), in which species the response of the nictitating membrane was recorded. Blood pressure was simultaneously recorded in this experiment.

The effect on neuromuscular transmission was also observed in cats similarly anesthetized but in which the sciatic nerve was electrically stimulated, and the contractions of the anterior tibial muscle were recorded by a myograph.

TOXICOLOGY

The *acute toxicity* of the hydrazine derivatives of isonicotinic acid was determined in mice by the oral, subcutaneous, intramuscular, intravenous, and intraperitoneal routes of administration. Observations of acute toxicity were also made in rats, rabbits, and dogs. In these last two species, the isopropyl derivative (Marsilid) was also administered intrathecally.

Subacute toxicity determinations were carried out by the daily oral or intravenous administration of relatively large doses of the two drugs to dogs over a short period of time. Limited pathologic observations were made in these animals. In these tests the isopropyl derivative (Marsilid) was administered as a solution of the dihydrochloride, but the dosage is expressed as the free base.

Chronic toxicity tests with concurrent hematologic studies were carried out in dogs in which the two drugs were administered by various routes, five days each week for thirteen weeks. Similar long-term studies were carried out in rats, but in this species the drug was incorporated in a ground diet so that its ingestion was continuous. In this latter test, the isopropyl derivative (Marsilid) was used in the form of the crystalline dihydrochloride. The dogs on the chronic toxicity experiment received a solution of the base, prepared

[4] These studies were carried out under the direction of Dr. L. O. Randall.

either from the dihydrochloride by the addition of sodium hydroxide or from the crystalline free base itself. The pH of these solutions was approximately 6.7. The dosage is expressed as mg. of the free base per kg. Isonicotinic acid hydrazide (Rimifon) was administered in all cases as the free base, either in the form of the crystalline powder or as a solution with a pH similar to that of the isopropyl hydrazine derivative (Marsilid).

Results

GENERAL PHARMACOLOGY

Isolated intestine: Tests for various types of pharmacologic activity have demonstrated that the hydrazine derivatives of isonicotinic acid exert few pharmacodynamic effects in a dosage of 1 to 10 mg. per kg. It was found that, on isolated rabbit intestine, isonicotinic acid hydrazide (Rimifon) showed less than 1/100 and the isopropyl hydrazine (Marsilid) less than 1/200 the activity of atropine in counteracting the hypertonic state induced by acetylcholine, and extremely high concentrations were necessary to inhibit peristalsis. In the dog with a Thiry-Vella fistula, irregular effects were produced by the intravenous administration of either agent. Tests for antihistamine activity on isolated guinea pig intestine indicated an activity less than 1/1,000 of that of β-dimethylaminoethyl benzhydryl ether hydrochloride (Benadryl) for both these agents.

Antisalivation: In the antisalivation tests in rabbits, doses as high as 25 mg. of isonicotinic acid hydrazide (Rimifon) per kg. or 50 mg. of the isopropylhydrazine (Marsilid) per kg. given subcutaneously did not suppress salivation; and lacrimation in the rat was not diminished by subcutaneous doses of these agents as high as 50 mg. per kg. The local application of 1.0 per cent solutions of either drug to the eyes of the rabbit did not induce either mydriasis or local anesthesia.

Bronchiolytic test: In the bronchiolytic aerosol test, 1 mg. of isonicotinic acid hydrazide (Rimifon) per kg. intraperitoneally had the same effect as 0.05 mg. of atropine per kg. Thus, in this test, isonicotinic acid hydrazide may be considered to be approximately one-twentieth as active as atropine. Surprisingly, the isopropyl derivative (Marsilid) was active at less than 0.001 mg. per kg., and in this respect has approximately fifty times the activity of atropine. In the histamine aerosol test, however, neither of these compounds showed activity when compared with the commonly used antihistaminics, nor were they active in relaxing isolated tracheal rings at a concentration of 10^{-4} gm. per cc.

Antipyretic activity: Tests for antipyretic activity revealed that isonicotinic acid hydrazide (Rimifon) in the dosage of 2 and 4 mg. per kg. and the isopropyl derivative (Marsilid) in the dosage of 4, 8, and 16 mg. per kg. had no effect in decreasing temperatures elevated in rabbits by the administration of *E. coli*.

Blood pressure: In blood pressure studies in the dog, intravenous doses of the hydrazides as high as 8 mg. per kg. had neither pressor nor depressor effects. Similarly, no effect upon the normal response to vagus stimulation, carotid occlusion, or injections of acetylcholine and of epinephrine was observed. In the cat, intravenous doses of 8 mg. of the isopropyl derivative (Marsilid) per kg. generally caused a depressor action varying up to 25 mm. of mercury of a few minutes' duration. The response to similar doses of isonicotinic acid hydrazide was

somewhat less. In these same animals, very little, if any, ganglionic blocking activity could be observed. There likewise was no effect on neuromuscular transmission in the cat.

TOXICOLOGY

Acute Toxicity

Mice: The acute toxicity in mice was investigated by administration of the materials by various routes. It involved the use of 324 mice. From the data presented in table 1, it is apparent that there was little difference in the toxicity when either agent was given by different routes, although the oral route appeared

TABLE 1

ACUTE TOXICITY OF ISONICOTINIC ACID HYDRAZIDE (RIMIFON) AND 1-ISONICOTINYL-2-ISOPROPYL HYDRAZINE (MARSILID)

ROUTE	ISONICOTINIC ACID HYDRAZIDE LD_{50} MG. PER KG.		1-ISONICOTINYL-2-ISOPROPYL HYDRAZINE LD_{50} MG. PER KG.	
	1 Day*	1 Week*	1 Day*	1 Week*
Mice				
Oral	142 ± 10.0	133 ± 14.0	968 ± 29.0	950 ± 28.5
Subcutaneous	160 ± 11.2	177 ± 19.5	750 ± 60.0	730 ± 65.7
Intravenous	153 ± 16.8	153 ± 16.8	725 ± 36.3	719 ± 36.0
Intraperitoneal	132 ± 13.2	130 ± 9.1	690 ±	690 ±
Intramuscular	140 ± 15.4	137 ± 15.1	683 ± 47.8	615 ± 61.5
Rat				
Oral		1,435		383
Rabbit				
Oral	250	200	150	125
Intravenous	94	94	150	117

* Observation period.

to be slightly less toxic than other routes in the case of the isopropyl derivative (Marsilid). This was not found to be the case with isonicotinic acid hydrazide (Rimifon).

A comparison of the LD_{50} values in mice of the two compounds, when these agents were given by identical routes, indicated that the isopropyl derivative (Marsilid) was 4.5 to 7.0 times less toxic than isonicotinic acid hydrazide (Rimifon). Mice receiving toxic doses of isonicotinic acid hydrazide (Rimifon) developed clonic convulsions which terminated in tonic limb extension and death by respiratory failure. Convulsions also occurred with the isopropyl derivative (Marsilid). Most of the deaths occurred within twenty-four hours after administration of the compounds.

Rats and rabbits: In rats and rabbits isonicotinic acid hydrazide (Rimifon) was

found to be less toxic than the isopropyl hydrazine derivative (Marsilid). The difference in toxicity was not great in rabbits, but in rats isonicotinic acid hydrazide was several times less toxic than the isopropyl derivative. Convulsive activity was also prominent in these species. Death was due to respiratory failure.

Dogs: In dogs, to which species intravenous doses of 50 and 100 mg. of isonicotinic acid hydrazide (Rimifon) per kg. were administered, signs of toxicity were delayed approximately one-half hour, at which time salivation, retching, vomiting, and defecation occurred. At the 100 mg. per kg. level this was followed by the development of central nervous system signs consisting of apprehension, anxiety, and convulsions which became continuous and terminated in death. The oral administration of isonicotinic acid hydrazide (Rimifon) produced a type of toxicity similar to that produced by its intravenous administration. When the isopropyl derivative (Marsilid) was given orally or intravenously to dogs, signs of toxicity were not noted until doses of 70 to 140 mg. per kg. (expressed as free base) were administered.

Intrathecal administration to rabbits and dogs: In addition to the above studies, the isopropyl derivative (Marsilid) was administered intrathecally to rabbits and dogs. In both species atlanto-occipital taps were carried out without asepsis after local infiltration of the area with 1 per cent procaine. Cerebrospinal fluid in amounts equivalent to the volume of injected material was permitted to flow out before the drugs were administered. In rabbits, volumes of 0.1 and 0.5 cc. of the isopropyl derivative (Marsilid) were given as a neutralized 5 per cent solution of the dihydrochloride. In dogs, volumes of 0.25, 0.5, 1.0, and 2.0 cc. of the same material were administered. Surviving animals were observed for a period of four weeks and were then sacrificed. The rabbits showed no respiratory, cardiac, or neurologic effects, and survived an observation period of four weeks. No signs of local pathology were evident when these animals were sacrificed and autopsied.

In the 4 dogs injected intrathecally, no immediate signs of respiratory, cardiac, or neurologic changes were evident. The dogs on the two higher doses died, however, after periods of twelve and twenty-two days. In one animal, adhesions were noted between the dura and the cortex just lateral to the midline. An autopsy of the second animal, which received the highest dose, was not carried out because of the marked post-mortem changes present when the animal was found dead.

Subacute Toxicity

In these studies, isonicotinic acid hydrazide (Rimifon) was administered orally to a group of 4 dogs with an initial dose of 50 mg. per kg. One of these animals died, but the remaining 3 were continued on a daily dosage of 25 mg. per kg. At the end of five days, blood was drawn from each of these dogs for hemoglobin studies because it had been noted previously that the blood of animals receiving isonicotinic acid hydrazide had a purplish hue. Values for total, met-, sulf-, and oxy-hemoglobin obtained by the method of Evelyn and Malloy (5) are reported in table 2. It is apparent from the values in this table that the oxyhemoglobin,

as well as the total hemoglobin, of the blood of these dogs was significantly reduced. Although the methemoglobin values in each of the three animals were increased and the sulfhemoglobin values were elevated in 2 of the dogs, these changes are not considered significant. These animals expired within a period of two weeks. Others on a dosage of 20 mg. per kg. survived thirteen weeks (*See Chronic Toxicity* below).

TABLE 2

ALTERATION IN THE HEMOGLOBIN CONTENT OF BLOOD OF DOGS ON HYDRAZINE DERIVATIVES OF ISONICOTINIC ACID

DOG NUMBER	DOSE	GRAMS HEMOGLOBIN/100 CC. BLOOD			
		Total	Met-	Sulf-	Oxy-
Isonicotinic Acid Hydrazide (Rimifon) (five daily oral doses; initial dose 50 mg/kg, then 25 mg/kg)					
	mg/kg				
1A	25+	Before 14.70	0.256	0.109	14.35
		After 11.85	0.298	0.063	11.51
3A	25+	Before 13.57	0.053	0.178	13.38
		After 12.39	0.285	0.230	11.92
4A	25+	Before 14.45	0.126	0.034	14.30
		After 12.89	0.220	0.224	12.49
1-Isonicotinyl-2-Isopropyl Hydrazide (Marsilid) (five daily intravenous doses)					
4B	28	Before 12.80	−0.170	0.112	12.83
		After 3.25	0.143	0.578	2.66
5B	28	Before 13.06	−0.021	−0.085	13.15
		After 4.24	0.186	0.565	3.61
Controls					
6B	—	Initial 10.92	−0.083	−0.122	10.67
		Final 12.60	0.043	−0.040	12.61
7B	—	Initial 9.90	−0.065	−0.008	9.95
		Final 12.08	0.043	−0.077	12.12

When administered repeatedly to 2 dogs in a dose of 28 mg. per kg., the isopropyl derivative (Marsilid) induced evident anemia after five intravenous injections. In these animals, the average hemoglobin value fell from 13.0 to 3.8 gm. per 100 cc., with a corresponding reduction of the erythrocyte count from 4.4 to 2.1 million per cu. mm. Accompanying these changes were elevations in the reticulocyte count, leukocyte count, and urinary urobilinogen. The sulfhemoglobin concentrations of the blood, but not the methemoglobin concentrations, were significantly elevated in these animals (table 2). Dogs which received 56 mg. per kg. intravenously gave evidence of developing anemia after two injections and died after four injections. While dogs receiving 14 mg. per kg. survived thirteen weeks of treatment, a minor degree of anemia was evident after four

weeks of treatment. The anemic condition improved, however, as indicated by successive monthly hemograms (*See Chronic Toxicity* below).

Chronic Toxicity

Isonicotinic acid hydrazide (Rimifon) in rats: Chronic toxicity studies on isonicotinic acid hydrazide (Rimifon) were carried out in an experiment in which groups of 10 weanling male Wistar rats were placed upon levels of 0.001, 0.005, 0.02, and 0.05 per cent of the drug incorporated in the ground diet. Twenty animals served as controls. In figure 1, it may be seen that 0.001 and 0.005 per cent concentrations of isonicotinic acid hydrazide in the diet had no influence on

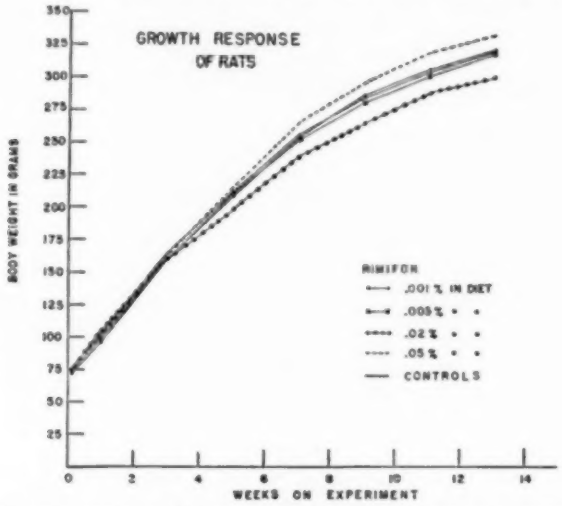

FIG. 1. Growth response of Wistar male rats on isonicotinic acid hydrazide (Rimifon) in the diet.

growth. Although 0.02 per cent appeared to have depressed growth, this effect cannot be considered significant because a higher concentration, i.e., 0.05 per cent, in the diet appeared to have stimulated growth.

In none of the groups were the erythrocyte counts, hemoglobin, or hematocrit values significantly altered. Only in the group on the highest level of isonicotinic acid hydrazide (Rimifon) was the average leukocyte count depressed after twelve weeks of drug administration (table 3). However, the differential count was not altered either in this group or in the others. When these animals were sacrificed, consistent gross pathology related to the drug was not apparent.

Isonicotinic acid hydrazide (Rimifon) in dogs: In long-term toxicity studies in dogs, isonicotinic acid hydrazide (Rimifon) was administered intravenously to each of 3 dogs at dose levels of 1, 5, and 20 mg. per kg., five days per week for

thirteen weeks. While the dog on 1 mg. per kg. exhibited no signs of toxicity, the dog on 5 mg. per kg. occasionally exhibited anorexia. The animal on 20 mg. per kg., in addition, had occasional emesis, failed to gain weight, and often appeared lethargic. In none of the animals were the final erythrocyte counts, hemoglobin, and hematocrit values significantly altered (table 4). No changes in liver function were observed as determined by bromsulfalein tests. Urinalysis revealed no significant change in urinary function. When sacrificed and autopsied, the dogs on 5 and 20 mg. per kg. showed gross evidence of slight liver pathology. This was not evident in the dog at the lowest dose level.

TABLE 3

AVERAGE BLOOD COUNTS OF RATS WHICH RECEIVED ISONICOTINIC ACID HYDRAZIDE (RIMIFON)*

NUMBER OF WEEKS ON EXPERIMENT	DOSE LEVEL IN DIET	TOTAL LEUKOCYTES IN THOUSANDS	ERYTHROCYTES IN MILLIONS	OXYHEMOGLOBIN	HEMATOCRIT PER CENT VOLUME ERYTHROCYTES	DIFFERENTIAL COUNT				
						Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
	per cent			*gm/100 ml*						
0	0.001	10.1	6.0	11.9	38.5	33	60	6.8	0.6	0.2
4		13.9	7.7	14.1	40.2	15	82	2.2	1.0	0.0
8		13.3	8.7	15.9	45.3	18	79	1.6	1.0	0.2
12		15.5	8.6	15.2	45.6	27	69	1.2	1.6	0.4
0	0.005	9.4	6.2	12.0	37.7	36	58	4.6	1.4	0.0
4		14.0	7.4	13.2	38.6	27	71	1.2	0.4	0.0
8		14.9	8.4	16.0	46.3	19	78	1.6	0.4	0.0
12		17.0	8.4	14.9	45.3	34	62	1.6	3.0	0.0
0	0.02	9.6	6.0	12.8	39.4	28	65	6.2	0.8	0.0
4		13.9	7.8	14.5	42.7	28	70	1.0	1.4	0.2
8		14.8	8.5	15.9	45.8	20	80	0.4	0.0	0.0
12		15.6	8.8	15.4	46.6	35	62	2.2	0.4	0.0
0	0.05	8.4	6.3	12.1	37.8	24	70	5.4	0.4	0.0
4		13.1	6.8	14.1	41.8	15	84	1.2	0.2	0.0
8		14.3	8.6	15.8	44.0	18	79	2.0	0.8	0.4
12		11.8	7.9	15.4	45.9	24	73	1.5	0.8	0.0
0	Controls	8.1	6.4	12.0	37.0	26	71	1.9	0.9	0.3
4		11.6	7.6	14.3	42.4	30	65	3.0	1.4	0.1
8		13.6	7.2	15.1	45.7	22	76	1.7	0.4	0.0
12		15.0	7.8	15.4	46.6	29	68	1.3	1.0	0.0

* Average of 5 animals on each dose of isonicotinic acid hydrazide and 9 control animals

1-Isonicotinyl-2-isopropyl hydrazine (Marsilid) in rats: Chronic toxicity studies on the isopropyl hydrazine derivative (Marsilid) were carried out in an experiment in which groups of 20 rats were placed upon oral dose levels of 0.005, 0.02, and 0.08 per cent of the dihydrochloride in the diet. Twenty animals also served as controls. The animals on 0.02 per cent and 0.08 per cent in the diet failed to

TABLE 4

BLOOD COUNTS OF DOGS WHICH RECEIVED HYDRAZINE DERIVATIVES OF ISONICOTINIC ACID

NUMBER OF WEEKS ON EXPERIMENT	DOSE LEVEL	TOTAL LEUKOCYTES IN THOUSANDS	ERYTHROCYTES IN MILLIONS	OXYHEMOGLOBIN	HEMATOCRIT PER CENT VOLUME ERYTHROCYTES	DIFFERENTIAL COUNT				
						Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Isonicotinic Acid Hydrazide (Rimifon)*										
				gm/100 ml						
0	1 mg/kg	19.8	5.2	13.6	44.0	84	10	2	4	0.0
4		19.2	5.6	13.6	43.0	80	5	5	11	0.0
8		26.0	6.8	16.0	43.2	79	6	4	11	0.0
12		19.8	7.5	17.5	50.0	71	6	6	17	0.0
0	5 mg/kg	17.0	4.8	12.8	38.2	67	12	3	17	1.0
4		22.3	4.9	12.8	38.0	87	9	2	2	0.0
8		14.2	6.0	14.6	41.9	71	14	7	8	0.0
12		9.3	5.6	15.1	39.5	75	12	3	9	1.0
0	20 mg/kg	17.0	5.3	12.8	36.2	74	13	1	12	0.0
4		12.7	4.7	12.6	39.0	84	8	3	4	1.0
8		17.2	5.7	14.4	41.8	77	18	2	1	2.0
12		13.3	6.2	14.7	42.5	56	28	5	11	0.0
1-Isonicotinyl-2-Isopropyl Hydrazine (Marsilid)†										
0	3.5 mg/kg	15.6	6.0	14.1	43.0	68	20	5	7	0.1
5		12.7	5.4	14.6	42.3	66	26	1	8	0.1
9		16.6	6.3	14.4	42.8	66	20	7	8	0.5
12		17.1	6.0	14.6	43.0	68	19	6	7	0.3
0	7.0 mg/kg	14.1	5.5	13.9	42.6	66	21	5	8	0.0
5		16.5	5.2	14.0	42.4	65	26	1	8	0.1
9		21.3	5.1	11.9	35.6	73	18	3	5	0.5
12		16.8	5.0	12.8	38.5	66	26	4	6	0.3
0	14.0 mg/kg	13.0	5.9	13.8	40.2	61	31	2	6	0.1
5		17.1	3.7	9.3	29.9	71	23	1	5	0.0
9		26.7	4.4	11.0	33.9	71	21	2	6	0.3
12		16.6	4.9	12.6	39.4	66	21	4	8	0.5
0	Controls‡	32.3	5.3	12.8	36.9	73	19	4	4	0.0
5		14.2	5.4	14.3	42.6	73	21	1	5	0.0
9		19.9	6.2	14.4	40.9	78	17	2	2	0.3
12		14.7	5.9	14.9	42.9	62	26	5	6	0.3

* Single animal on each dose

† Average of 6 animals on each dose

‡ Average of 3 animals

gain weight as rapidly as the controls during the first two weeks of feeding (figure 2). Thereafter, the rate of growth paralleled that of the controls, although the average weights were respectively 7 and 15 per cent below normal. Rats on

0.005 per cent of the isopropyl derivative (Marsilid) in the diet showed no significant depression of growth. Hematologic studies showed a decrease in erythrocyte and leukocyte counts and hemoglobin values of moderate degree with the two higher dose levels (table 5). At the highest dose level, a few nucleated erythrocytes were observed in the smears. The animals in this group, when picked up, also characteristically held their hind limbs in a position of semiflexion. At the lowest dose level, the average erythrocyte count, average hemoglobin value, and posture remained normal.

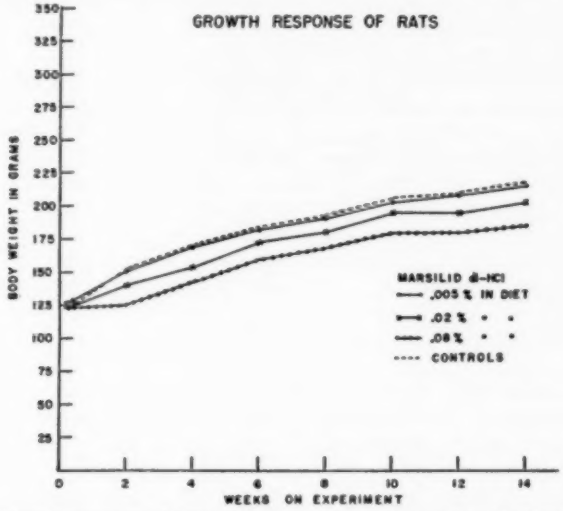

FIG. 2. Growth response of Sprague-Dawley female rats on 1-isonicotinyl-2-isopropyl hydrazine (Marsilid) in the form of the dihydrochloride in the diet.

After thirteen weeks on the drug, the rats were sacrificed and autopsied. The tissues of the rats on the lowest dose level appeared essentially normal. Those on the intermediate level presented spleens that were darker than normal. The external surfaces were somewhat rough and the cut surfaces were granular. The livers were pale brown in color. This pathology was accentuated on the highest dose level. In addition, the spleens were larger than normal. The kidneys of rats on the highest level showed slightly diminished corticomedullary differentiation. Renal pigmentation also was present in rats on the high dose.

1-Isonicotinyl-2-isopropyl hydrazine (Marsilid) in dogs: In a chronic toxicity determination in dogs, the isopropyl hydrazine derivative (Marsilid) was administered at dose levels of 3.5, 7.0, and 14.0 mg. per kg. by way of the oral, intravenous, and intramuscular routes. Six animals were placed on each of the three dose levels with 2 animals receiving the drug by each of the routes of administration. Three animals served as controls and received saline by the three

respective routes. A total of 21 dogs was used in this experiment. All survived the thirteen-week period of drug administration without physical evidence of intolerance or toxicity. The treated animals gained weight comparable with the controls and remained in a state of apparent good health throughout the experiment. Animals on the 3.5 mg. per kg. dose level showed no reduction in average erythrocyte counts, hemoglobin, or hematocrit values. The animals on 7.0 mg. per kg. showed a slight reduction in all of these values, while dogs on 14.0 mg. per kg. presented a significant reduction in these, particularly at the end of one month's treatment. In addition, an increased number of nucleated erythrocytes

TABLE 5

AVERAGE BLOOD COUNTS OF RATS WHICH RECEIVED ISONICOTINIC ACID HYDRAZIDE (MARSILID)*

NUMBER OF WEEKS ON EXPERIMENT	DOSE LEVEL IN DIET	TOTAL LEUKOCYTES IN THOUSANDS	ERYTHROCYTES IN MILLIONS	OXYHEMOGLOBIN	DIFFERENTIAL COUNT				
					Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
	per cent			*gm/100 ml*					
0	0.005	16.0	6.1	12.7	22	76	1.3	0.3	0.2
6		17.8	8.4	14.6	11	86	0.9	2.3	0.0
12		14.4	7.5	15.3	12	87	1.2	0.2	0.0
0	0.02	15.3	5.7	12.8	18	80	1.4	0.4	0.1
6		17.1	7.3	14.6	10	87	1.6	1.4	0.2
12		15.0	6.6	14.0	13	86	0.3	1.0	0.0
0	0.08	15.0	5.8	12.3	18	81	0.4	0.7	0.2
6		17.5	6.6	13.4	12	84	1.5	0.6	0.8
12		15.7	6.2	12.4	11	88	0.9	0.5	0.0
0	Controls	14.0	5.8	12.6	20	78	0.9	0.3	0.6
6		14.6	8.1	15.4	16	81	2.1	1.3	0.2
12		18.6	7.3	15.2	11	87	0.9	1.2	0.1

* Average of 10 animals on each dose

were observed in the blood smears. All of these values gradually improved, as noted by successive monthly hemograms, so that by the end of twelve weeks they were not markedly reduced when compared with the control animals (table 4). Coagulation times were in the normal range.

When autopsied, however, all animals which received the isopropyl derivative (Marsilid) presented gross pathology. This was most manifest in the spleens, bone marrow, livers, and kidneys of animals on the highest dose. The spleens were dark, enlarged, and firm. The bone marrow was hemorrhagic. The livers were pale brown and the kidneys presented a fibrosis with diminished corticomedullary differentiation. The latter pathology was not evident at the lowest dose, i.e., 3.5 mg. per kg., but slight alterations were noted in the spleens, bone marrow, and livers. Intermediate degrees of pathology were observed in animals on the

7.0 mg. per kg. level. Sections of muscle taken from the site of injection in the dogs treated intramuscularly presented a dark and fibrotic appearance. Likewise, sections of vein from the animals treated intravenously were fibrotic, but no gastrointestinal changes were evident in the dogs treated orally.

Discussion

The pharmacologic study of the hydrazides here reported was instituted when it was found that these compounds exhibited marked antituberculous activity. The toxicologic studies have served as a guide in the selection of clinical dosage, and the pharmacodynamic studies have provided information subsequently helpful in excluding the possibility that the clinical improvement of patients was a consequence of causes other than the specific chemotherapeutic effects of the drugs (6). In addition, the present studies have served to explain, in part, mechanisms involved in the appearance of certain clinical side effects.

The lack of general pharmacodynamic properties of the hydrazides, in dosages which have ameliorative effects in tuberculosis, suggests that these agents would be free of side effects. Selikoff, Ornstein, and Robitzek (7), however, report that a number of patients receiving 1 to 10 mg. per kg. of 1-isonicotinyl-2-isopropyl hydrazine (Marsilid) experience dizziness, insomnia, constipation, or urinary bladder retention, the last two effects being overcome by administration of Prostigmine.[5] This led them to suggest that these effects might be of autonomic origin and due to either parasympathetic blocking or sympathetic stimulating properties of the drugs.

In the many tests carried out to detect possible parasympathetic blocking action, atropine-like activity of these compounds could not be elicited even in doses far exceeding those used clinically. An exception to this, however, was the striking bronchiolytic activity found against the cholinergically induced bronchospasm in guinea pigs. In this respect, the isopropyl derivative (Marsilid), the more active of the two compounds, was found to be fifty or more times as active as atropine in counteracting the spasm induced by an aerosol of methacholine. This bronchiolytic activity was not manifest, however, when bronchospasm was induced by an aerosol of histamine.

In contrast to the unusual antimethacholine activity, evidence for an atropine-like activity as the basis for the clinical report of constipation has not been found. On isolated strips of intestine, isonicotinic acid hydrazide and the isopropyl derivative in concentrations five hundred times greater than atropine had no effect in counteracting the spasm induced by acetylcholine. Only in extremely high concentrations did these compounds inhibit normal peristalsis. In tests for atropine-like activity on the pupil and the salivary glands, doses of the hydrazides five hundred times as great as atropine had no effect. On the basis of the inability of these compounds to suppress salivary secretion, one is inclined to interpret the clinical effect of sputum reduction in the tuberculous patient as a real chemotherapeutic effect of the drug on the tuberculous lesion and not just

[5] Proprietary name, Hoffmann-LaRoche, Inc., for neostigmine.

an effect upon general bronchial secretion. The reported change in the character of the sputum would also tend to confirm this point of view.

The clinical report (6) of dizziness in patients receiving the isopropyl derivative (Marsilid) conceivably could be due to changes in blood pressure. In anesthetized dogs, the intravenous administration of doses up to 8 mg. per kg. produced neither pressor nor depressor effects. In the cat, however, similar dosage did produce a transient depressor action, and such a response could occur in the human. The possibility that the symptom of dizziness is related to the central stimulating action of these drugs has not been ruled out, however. The overalertness and insomnia experienced by some patients receiving the isopropyl derivative (Marsilid) is suggestive of this possibility. Only at extremely high oral or intravenous doses in unanesthetized dogs, however, were signs of central nervous system stimulation evident. The survival of rabbits and dogs without neurologic signs when these agents were administered intrathecally speaks against a direct effect upon the central nervous sytem. Whether or not a direct stimulation of the sympathetic nervous system is involved still needs to be more adequately investigated.

The similarity of LD_{50} values in mice when either of the hydrazides was administered by several routes points to the completeness of absorption of these compounds in this species. That the degree of absorption may be variable from one species to another when administered orally is suggested by the fact that, while the isopropyl derivative (Marsilid) is many times less toxic than isonicotinic acid hydrazide (Rimifon) in the mouse, it is more toxic than isonicotinic acid hydrazide in the rat and rabbit. Furthermore, when given intravenously to the rabbit, the isopropyl compound (Marsilid) is less toxic than isonicotinic acid hydrazide (Rimifon).

With repeated doses of 25 mg. or more of either hydrazide per kg., changes in the hemoglobin characteristics of the blood were noted. At these doses, significant reductions in the total hemoglobin as well as the oxyhemoglobin content of the blood of dogs were observed. Associated with these changes, it was found that the sulfhemoglobin content of the blood was increased with Marsilid. More prolonged administration of the isopropyl derivative in high dosage led to the development of a hypochromic anemia. That this anemia was not of an aplastic type was substantiated by elevations in the leukocyte count, reticulocyte count, and urinary urobilinogen concomitant with the fall in erythrocyte count and total hemoglobin value. The urine of these animals was colored and gave a positive test for urobilin but not for bilirubin. At autopsy, the livers of these animals were pale, and the bile was dark in color.

Although the erythrocytic destructive property of phenylhydrazine and acetylphenylhydrazine has been used to clinical advantage in the treatment of polycythemia vera since their respective introductions, it is claimed by Allen and Page (8) that hydrazine itself does not possess this property. Although the compounds here reported are hydrazine derivatives of isonicotinic acid, the chemical relation to hydrazine does not, therefore, necessarily imply that they have the same effect on the erythrocyte as phenylhydrazine or acetylphenylhydrazine.

The oral chronic toxicity studies in rats have indicated that isonicotinic acid hydrazide (Rimifon) is less toxic than the isopropyl derivative (Marsilid). Although the two agents were not directly compared in the same group of animals, it was found that at a dose level of 0.02 per cent in the diet isonicotinic acid hydrazide exhibited no deviation from the normal in terms of growth, the blood picture, or the gross appearance of tissues. With the isopropylhydrazine (Marsilid), all of these were altered. The microscopic examination of the tissues from the rats on isonicotinic acid hydrazide has shown, however, that pathology does occur at this dose level. This was manifest primarily as an increased cellularity of the bone marrow with a relatively diminished number of adult neutrophils, slight hyperemia of the spleen, and slight parenchymatous degeneration of the liver, kidneys, and testes. The changes in the latter organs did not appear irreversible and may have been related to an intercurrent *Salmonella* infection.

In the chronic feeding experiments in rats, evidence for a "growth stimulant action" was not observed. This is of interest because it indicates that the weight gain achieved by tuberculous patients receiving these agents is related to an increase in appetite secondary to a reduction in the toxicity of the disease rather than to a direct stimulation of the appetite by these compounds. It also tends to indicate that the weight gain in patients is not related to tissue imbibition of fluids by these drugs.

The chronic toxicity studies carried out in dogs on isonicotinic acid hydrazide (Rimifon) and the isopropyl derivative (Marsilid) were not identical, but the isopropyl derivative appeared somewhat less toxic than isonicotinic acid hydrazide in so far as weight gain and general observations were concerned. On the basis of gross pathologic changes in tissues, the reverse situation appeared to exist. The hemorrhagic bone marrow noted in dogs receiving the isopropyl derivative (Marsilid) was not noted in dogs receiving comparable doses of isonicotinic acid hydrazide. The degree of reversibility of the bone marrow, hepatic, splenic, and renal changes is under investigation.

Summary

The hydrazine derivatives of isonicotinic acid (isonicotinic acid hydrazide [Rimifon] and 1-isonicotinyl-2-isopropyl hydrazine [Marsilid]) exert minor pharmacodynamic effects in animals when administered in single small doses. With the exception of a marked bronchiolytic effect exerted by the latter compound against cholinergically induced bronchospasm in the guinea pig, little evidence is available for autonomic activity of a parasympathetic blocking type.

Single doses in the acutely toxic range induce signs of central nervous system stimulation terminating in death due to respiratory failure. In mice and dogs, 1-isonicotinyl-2-isopropyl hydrazine (Marsilid) is less toxic than isonicotinic acid hydrazide (Rimifon). When administered orally to rats and rabbits, isonicotinic acid hydrazide (Rimifon) is less toxic than the isopropyl compound (Marsilid). When repeatedly administered in high dosage to dogs, alteration in the hemoglobin characteristics of the blood occurs.

The chronic administration of low concentrations of these agents in the diet of rats has no deleterious effect upon growth, erythrocyte counts, hemoglobin values, or the gross appearance of tissues. The chronic administration of low doses of these agents to dogs over a period of thirteen weeks is moderately well tolerated, but pathologic changes in reticulo-endothelial organs are induced.

Sumario

Observaciones Farmacológicas y Toxicológicas sobre los Derivados Hidracínicos del Acido Isonicotínico (Rimifon, Marsilid)

Los derivados hidracínicos del ácido isonicotínico, hidracida del ácido isonicotínico (Rimifón) y 1-isonicotinil-2-isopropil hidracina (Marsilid) ejercen efectos farmacodinámicos de menor cuantía cuando se administran a los animales en pequeñas dosis únicas. Exceptuado un notable efecto bronquiolítico ejercido por el segundo compuesto contra el broncoespasmo evocado colinérgicamente en el cobayo, pocas son las pruebas de actividad autónoma en forma de bloqueo del parasimpático.

Los dosis únicas en la escala de toxicidad aguda evocan signos de excitación del sistema nervioso central que culminan en la muerte debida a insuficiencia respiratoria. En los ratones y perros, la 1-isonicotinil-2-isopropil hidracina (Marsilid) es menos tóxica que la hidracida del ácido isonicotínico (Rimifón). Administrada oralmente a las ratas y los conejos, la hidracida (Rimifón) es menos tóxica que el compuesto isopropílico (Marsilid). Administrada repetidamente a dosis altas a los perros, sobrevienen alteraciones en las características de la hemoglobina en la circulación.

La adición crónica de esos agentes a bajas concentraciones en el alimento de las ratas no ejerció efectos nocivos sobre el crecimiento, las fórmulas eritrocitarias, los tenores de hemoglobina o el aspecto macroscópico de los tejidos. La administración crónica de los mismos a dosis bajas a los perros durante un período de trece semanas fué tolerada moderadamente bien, pero evocó alteraciones patológicas en los órganos retículo-endoteliales.

Resume

Remarque pharmacologique et toxicologique sur les dérivés hydrazines de l'acide isonicotinique (Rimifon, Marsilid)

Les dérivés hydrazinés de l'acide isonicotinique, hydrazide de l'acide isonicotinique (Rimifon) et 1-isonicotinyl-2-isopropyl hydrazine (Marsilid), exercent une action pharmacodynamique mineure chez l'animal, quand ils sont administrés à ceux-ci sous la forme de doses uniques faibles. Chez le cobaye, à l'exception d'une action bronchiolytique marquée, exercée par ce dernier composé contre le bronchospasme cholinergique provoqué, on ne dispose que de peu d'indications témoignant d'une action parasympathique du type parasympaticolytique.

La dose unique, dans la gamme des doses toxiques, provoque des signes d'excitation du système nerveux central, qui se terminent par la mort par inhibition de la respiration. Chez la souris et le chien, le 1-isonicotinyl-2-isopropyl hydrazine (Marsilid) est moins toxique que l'hydrazide de l'acide isonicotinique (Rimifon). Administrée par voie buccale au rat et au lapin, l'hydrazide de l'acide isonicotinique (Rimifon) est moins toxique que le composé isopropyl (Marsilid). Chez le chien, l'administration répétée, à hautes doses, provoque une altération des caractères de l'hémoglobine dans le sang.

A faibles concentrations, l'administration prolongée de ces composés dans le régime alimentaire du rat, n'a montré aucune action nocive sur la croissance, la numération globulaire, la valeur de l'hémoglobine ou l'aspect macroscopique des tissus. Chez le chien l'administration prolongée à faibles doses de ces composés, pendant une période de treize semaines, a été assez bien tolérée, mais des modifications pathologiques du système réticulo-endothélial ont été observées.

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PHARMACOLOGY OF ISONICOTINIC ACID HYDRAZIDE (NYDRAZID[1])[2]

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INTRODUCTION

Isonicotinic acid hydrazide (Nydrazid) was found to be highly active against experimental tuberculosis in mice by Bernstein and associates (1). Significant chemotherapeutic activity was found at drug diet levels corresponding to a daily intake of 2 mg. per kg., whereas the maximum acceptable level was found to be 64 mg. per kg. per day.

The present report includes acute, subacute, and chronic toxicity studies with isonicotinic acid hydrazide (Nydrazid) in mice, rats, and dogs. Plasma concentrations of the drug were determined at appropriate intervals in the chronic toxicity tests and in various acute studies.

EXPERIMENTAL OBSERVATIONS

Determination of Isonicotinic Acid Hydrazide (Nydrazid) in Plasma

Method: For the animal studies an initial rapid method was evolved for the determination of the isonicotinic acid hydrazide concentration in plasma by extracting with an equal volume of isoamyl alcohol and determining the ultraviolet absorption of the alcohol solution at 265 mu. An empirical curve of plasma concentration versus optical density was obtained from the absorption of alcoholic extracts of known concentrations of isonicotinic acid hydrazide in plasma corrected for plasma blanks. The error of determination of plasma concentrations of 5 γ per ml. or higher was ± 10 per cent. The minimum detection level was 3 γ per ml., but below 5 γ per ml. the error of determination was increased considerably. Improved procedures for the estimation of isonicotinic acid hydrazide in plasma and urine have been developed by Kelly and Poet (2).

In an attempt to estimate the blood concentration of isonicotinic acid hydrazide achieved in tests of effectiveness and acceptability by Bernstein and associates (1), mice were offered diets containing 2, 8, 32, and 128 times the minimum effective drug level. At intervals up to seventeen days mice were sacrificed, and pooled blood samples were submitted for drug analysis as outlined above. In no instance was a concentration of drug greater than 5.5 γ per ml. in plasma obtained.

Two mongrel dogs were utilized for plasma determinations of isonicotinic acid hydrazide after single oral doses of 4 and 20 mg. per kg. and one intravenous dose of 5 mg. per kg. Rapid absorption after oral dosage was indicated, since peak plasma drug concentrations were attained in thirty minutes or less (figure 1). At the end of five hours, the plasma drug concentration was at least 33 per cent of the concentration present one hour after administration.

[1] Proprietary name, E. R. Squibb & Sons.

[2] From the Squibb Institute for Medical Research, New Brunswick, New Jersey.

Acute Toxicity

Mice: A total of 135 albino mice ranging in weight from 18 to 24 gm. were employed for acute toxicity determinations. The acute oral, subcutaneous, and intravenous LD_{50} doses plus or minus standard error were 190 ± 6, 170 ± 5, and 165 ± 4 mg. per kg., respectively. Calculations of the LD_{50} and standard error were made according to the graphical method of Miller and Tainter (3). Nearly all deaths occurred within two hours after dosage; 2 deaths occurred at forty-eight hours and one death at ninety-six hours. Symptoms of toxicity were excitement, prostration, running movements, and convulsions, but none of these were observed in less than thirty minutes after administration of the drug by any route. Respiratory arrest occurred before cardiac arrest.

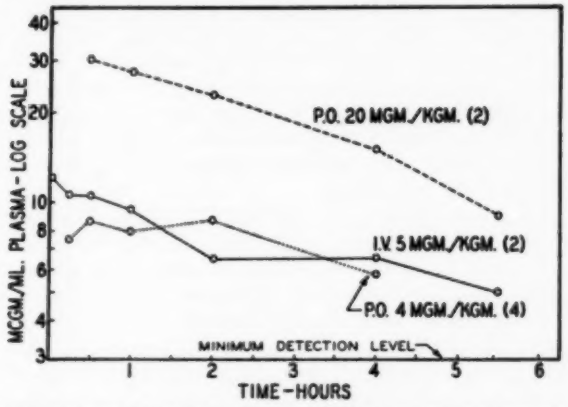

FIG. 1. Isonicotinic acid hydrazide (Nydrazid) plasma concentrations after oral and intravenous administration to 2 dogs. Each point is the average of the number of tests shown in parentheses.

Thirty other mice were given orally the estimated oral LD_5 dose, 144 mg. per kg., and cardiac blood samples were obtained one-half hour later for analysis. The mean drug plasma concentration was found to be 44 γ per ml.

Dogs: One unanesthetized dog received isonicotinic acid hydrazide, 100 mg. per kg., intravenously during a five-minute period. After thirty minutes the animal vomited and salivated excessively. Subsequently the animal was prostrated and exhibited tonic and clonic convulsions. Death occurred one hour and fifteen minutes after dosing. Normal values for erythrocyte, leukocyte, and differential leukocyte counts, hemoglobin, erythrocyte sedimentation rate, hematocrit, and methemoglobin were obtained at fifteen minutes, one hour, and at death. Similar results were obtained in a second dog injected with 50 mg. per kg. except that death did not occur until three hours after dosing.

A. P. Richardson (4) investigated the intravenous toxicity of isonicotinic acid hydrazide (Nydrazid) by single and repeated daily injection into 11 dogs. Evalu-

ation of these data, combined with those reported above, indicated an acute intravenous LD_{50} dose in dogs of approximately 50 mg. per kg. and an LD_{50} of approximately 22 mg. per kg. per day when dosage was repeated every twenty-four hours for two weeks.

Richardson (4) also administered the drug to one dog anesthetized with morphine and chloralose. When a cumulative total dose of 250 mg. per kg. was administered intravenously, hypotension, bradycardia, and irregular pulse were observed with the onset of vomiting and convulsive seizures, followed by death. Isonicotinic acid hydrazide was without effect on the typical blood pressure response to acetylcholine, epinephrine, and histamine.

Subacute Oral Toxicity

Regimens: Four groups of 5 albino rats ranging in weight from 100 to 120 gm. each were maintained on a stock diet and water *ad libitum*. One group was given water *per os* as a control and the other groups were dosed *per os* with isonicotinic acid hydrazide (Nydrazid) at 40, 160, and 640 mg. per kg. per day, divided into four doses for a maximum period of twenty-one days. A fifth group was given 320 mg. per kg. per day, divided into two doses, for a maximum period of fourteen days. Body-weight curves were obtained on all groups, and food consumption records were kept on the fifth group.

Body weight and food intake: In the first week, the groups of rats receiving the three upper dose levels lost weight, and the group receiving the lowest dose level failed to gain as rapidly as the controls. From this it was apparent that 40 mg. per kg. per day in the rat were about equivalent to the maximum acceptable dose level of 64 mg. per kg. per day in the mouse (1).

At the end of the twenty-one-day test period, the mean increases in body weight were 37, 35, and 13 gm. for the control group and the groups receiving 40 and 160 mg. per kg. per day, respectively. The higher dose groups manifested continual losses in body weight. After six days of the test, the 320 mg. per kg. per day group showed a mean decrease in body weight of 28 gm. and the 640 mg. per kg. per day group, a mean decrease of 20 gm. At 320 mg. per kg. per day, one rat died on the seventh day and another on the twelfth day; whereas one and 2 rats were sacrificed and autopsied on the ninth and fourteenth days, respectively. At 640 mg. per kg. per day, one rat was sacrificed after showing convulsive seizures on the seventh day, 2 more died on the tenth day, and the remaining 2 rats were sacrificed on the same day and autopsied. The food consumption record of the 320 mg. per kg. per day group showed that the body weight loss was correlated with the reduced food intake.

Post-mortem findings: In the 3 sacrificed rats receiving 320 mg. per kg. per day, the gross appearance of the heart, lungs, liver, kidneys, spleen, and pancreas was essentially normal. Microscopically, the heart, lungs, spleen, adrenals, and pancreas of these animals appeared to be normal. The liver of one of these 3 rats appeared normal, one showed two necrotic areas and slight granular degeneration, and the other slight fatty degeneration. The kidneys of these 3 test rats showed only slight granular degeneration of the proximal convoluted tubules. Similar microscopic lesions were not observed in 2 control rats examined.

Grossly, in the 2 sacrificed rats receiving daily oral doses of 640 mg. per kg. per day, there were no definite pathologic changes in the lungs, kidneys, heart, liver, spleen, pancreas, or adrenals. The tissues of one of these rats were examined microscopically and the pathologic changes noted were similar to those described above.

Dog Chronic Oral Toxicity

The dose levels for the dog chronic study were calculated from the maximum tolerated dose levels in mice and rats in the subacute experiments. Calculation of dosage in mg. per kg. of body weight and tolerance periods from one species to another on the basis of relative body surface area led to the estimates shown in table 1.

Groups of 2 mongrel dogs were studied at dose levels of 4, 10, and 25 mg. per kg. per day. When the highest dose proved to be rapidly toxic, a fourth dosage group of 2 dogs was initiated at 17.5 mg. per kg. per day.

TABLE 1

MAXIMUM TOLERATED DAILY ORAL DOSE OF ISONICOTINIC ACID HYDRAZIDE (NYDRAZID)

ANIMAL	DAILY DOSE (mg/kg)		MINIMAL TOLERATED PERIOD (WEEKS)	
	Calculated	Experimental	Calculated	Experimental
Mouse	—	64 Reference (1)	—	½
Rat	38	40	1	1
Dog	10	—	4	—

The dogs were given the drug orally at 8:00 a.m. and 4:00 p.m. daily except weekends when only the morning dose was administered. Such dosage was continued until death or sixteen weeks to date.[2] The dogs employed ranged in body weight from 8.7 to 13.2 kg. Each dog had been kept in the laboratory for at least three months before being placed on test, during which time the dogs were immunized, deparasitized, and control laboratory values were obtained.

Clinical Laboratory Tests

The routine determinations and observations on each dog before and during the test period consisted of the following biweekly examinations: hematologic and chemical studies (see below), plasma drug concentrations, urinalyses, and physical tests. The body weight was determined at approximately three-day intervals, and a daily record was kept of food consumption and animal symptomatology.

The hematologic studies consisted of erythrocyte and leukocyte counts, differential leukocyte counts, determinations of hematocrit, hemoglobin, sedimentation rate, and one-stage prothrombin time. Chemical tests of the blood included determinations of nonprotein nitrogen, urea nitrogen, dextrose, serum specific gravity, serum protein, cholesterol, creatinine, and the qualitative van den Bergh test for bilirubin. When indicated, the bilirubin tolerance was determined. Serum calcium and methemoglobin determinations were made

[2] Since preparation of the manuscript the test period has been extended to a total of twenty weeks without further changes in symptomalogy or laboratory findings in the 4 surviving dogs.

at irregular intervals. The physical tests included electrocardiograms with standard limb leads, basal metabolic rates, cuff blood pressures, and rectal temperatures. Urinalyses included determinations of albumin, reducing substance, specific gravity, pH, color, and microscopic examinations.

Twenty-five milligrams per kilogram per day: At the highest dose level, 25 mg. per kg. per day, anorexia, loss in body weight, ataxia, body tremors, tonic and

TABLE 2

GROSS AND MICROSCOPIC PATHOLOGIC CHANGES IN DOGS TREATED WITH ISONICOTINIC ACID HYDRAZIDE (NYDRAZID)

DOG NUMBER	ORAL DOSE MG/KG/DAY	TEST DAYS	ORGANS SHOWING PATHOLOGIC CHANGES*
9202	25	28	Found dead Liver: pronounced fatty degeneration (80 to 90%) Kidneys: slight to marked fatty degeneration of convoluted tubules Intestines: small subacute inflammatory mucosal ulcer (1 to 1.5 cm.) in duodenum; few petechiae in jejunum, cecum, and rectum Bone marrow: no smear made
9206	25	10	Sacrificed 43 days after last dose Liver: very few small sites of 5 to 20 parenchymal cells showing marked fatty degeneration; otherwise apparently normal
9208	17.5	31	Found dead Liver: pronounced fatty degeneration (80 to 90%) Kidneys: slight fatty degeneration of proximal convoluted tubules Stomach: contents greenish-brown fluid; otherwise normal Bone marrow: no smear made
9211	17.5	33	Found dead 6 days after last dose; generalized icterus Liver: appears to be regenerated (parenchymal cell pattern somewhat disorganized); considerable cellular debris in sinusoids Kidneys: only slight degeneration of proximal tubules and moderate amount of serous exudate in glomerular capsules Thyroid: slight to moderate desquamation of epithelial cells Bone marrow: myeloid erythroid ratio on 500 cells was 4.05 (normal range 1.45 to 3.0), primarily due to a decrease in the erythroid cells

* Heart, lungs, thyroid, stomach, liver, spleen, pancreas, intestines, adrenals, and bone marrow were examined and found normal unless noted otherwise.

clonic convulsions, prostration, and jaundice developed progressively within the first two to four weeks in both dogs. Peak convulsive activity occurred one to two hours after administration of the drug. One dog (No. 9202) died on the twenty-ninth day and was autopsied. When severe toxicity became apparent on the tenth day in the second dog (No. 9206), administration of the drug was stopped

and forced feeding was initiated. A rapid reversal of the symptoms occurred within the next two weeks. This animal was sacrificed and autopsied forty-three days after cessation of dosage.

Seventeen and five-tenths milligrams per kilogram per day: At the second highest dose level, 17.5 mg. per kg. per day, similar symptoms developed within the

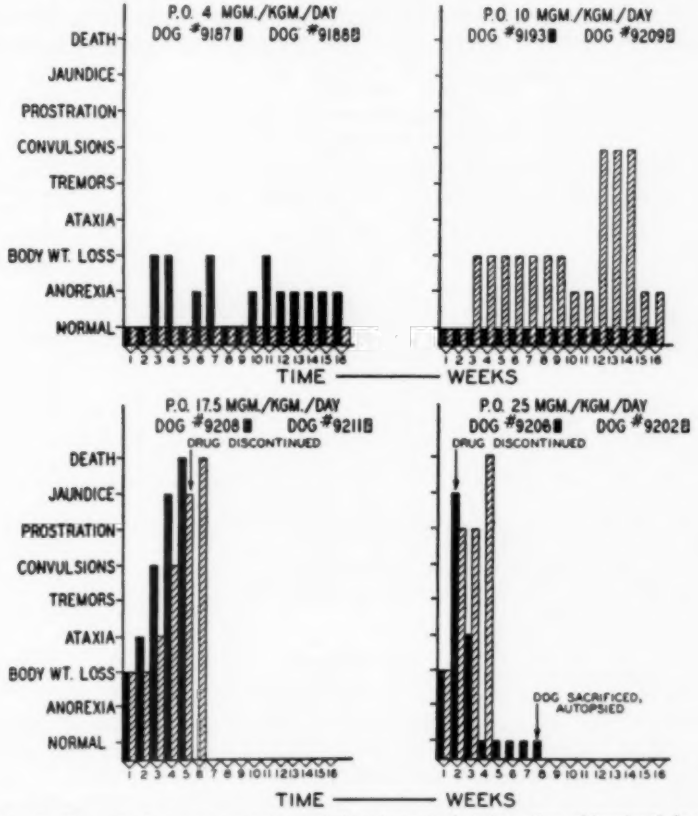

FIG. 2. Chronologic symptomatology of chronic oral administration of isonicotinic acid hydrazide (Nydrazid) to dogs.

first four to five weeks in both dogs. One dog (No. 9208) died on the thirty-second day; the other (No. 9211), on the thirty-ninth day in spite of cessation of drug administration after the thirty-third day. Both animals were autopsied. The gross and microscopic pathologic findings on the 4 autopsied dogs are summarized in table 2.

Ten milligrams per kilogram per day: At the dose level of 10 mg. per kg. per day, one dog (No. 9193) has been symptom free for sixteen weeks to date[3]

except for slight anorexia during the eighth and ninth weeks. During the first nine weeks the other dog (No. 9209) showed irregular periods of anorexia and a slight loss in body weight. In the next seven-week period, irregular and transient bouts of ataxia, body tremors, and tonic and clonic convulsions were observed in this second dog (No. 9209). No deaths or evidence of jaundice have occurred in this group to date.[3]

Four milligrams per kilogram per day: At the lowest dose level employed, 4 mg. per kg. per day, one dog (No. 9188) has been symptom free throughout

TABLE 3

CLINICAL LABORATORY CHANGES DETECTED IN DOGS DURING CHRONIC ORAL ADMINISTRATION OF ISONICOTINIC ACID HYDRAZIDE (NYDRAZID)

ORAL DOSE	DOG NUMBER	TEST DAYS	ERYTHROCYTES	HEMOGLOBIN	LEUKOCYTES		SERUM PROTEIN	PLASMA NPN	BILIRUBIN		HEART RATE	BASAL METABOLIC RATE
					Abs	Diff			Endogenous	Tolerance		
mg/kg/day												
4	9187	116	0	0	+	0	0	0	0	0	0	0
	9188	116	−	−	+	0	0	0	0	0	0	0
10	9193	116	−	−	0	0	0	0	0	0	0	0
	9209	116	0	0	+	0	0	0	0	0	0	+
17.5	9208	31	0	0	0	0	−	0	+	−	−	0
	9211*	33	0	0	0	0	−	0	++	=	−	+
25	9202	28	−	−	+	‡	−	0	++	=	−	+
	9206†	10	−	−	+	‡	−	+	++	=	−	0

* Drug administration stopped after thirty-third day, animal moribund; dead on thirty-ninth day.

† Drug administration stopped after tenth day; all values but the leukocyte count returned to normal within the following two weeks.

‡ Neutrophils increased, lymphocytes decreased.

++ = moderate to marked increase

\+ = slight increase

0 = no significant change

− = slight decrease

= = moderate to marked decrease

the sixteen-week test to date.[3] The second dog (No. 9187) evidenced mild anorexia and slight loss in body weight, but no evidence of jaundice or central nervous system disturbance has been observed.

Chronologic incidence of symptoms in all groups: The chronologic incidence of these various symptoms among the 8 dogs is summarized in figure 2. The 4 surviving dogs on the two lower dose levels are being continued on test.

The only noteworthy changes in hematology, blood chemistry, and physical tests detected among the 8 dogs are cited in table 3. Urinalyses and other clinical laboratory determinations not included in table 3 have not deviated significantly from normal.

Plasma concentrations: The plasma drug concentrations attained in the 8 dogs

during chronic oral administration were roughly proportional to the dose. As judged by plasma drug concentrations alone, no evidence of cumulation was detected, particularly at the three lower dose levels (table 4). The plasma drug concentrations of one dog (No. 9202) receiving 25 mg. per kg. per day were 4 and 31 γ per ml. thirty minutes before and after the morning dose, respectively, on the twenty-third day. Furthermore, when this same drug dosage was discontinued in the other dog (No. 9206) after the morning dose on the tenth day, the plasma concentration fell from 23 γ per ml. at 8:30 a.m. to 5 γ per ml. by 4:30 p.m. At 8:30 a.m. on the following day, no drug could be detected in the plasma.

TABLE 4

PLASMA CONCENTRATIONS OF ISONICOTINIC ACID HYDRAZIDE (NYDRAZID) DURING CHRONIC ORAL ADMINISTRATION IN DOGS

ORAL DOSE	DOG NUMBER	DRUG PLASMA CONCENTRATION (γ/ML)* (30 MINUTES AFTER DOSAGE)								Average per dog	Average per dose
		Weeks of Test									
		1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16		
mg/kg/day											
4	9187	4.2	4.0	4.0	–	–	4.5	4.6	4.5	4.3	4.1
	9188	4.2	3.8	4.6	–	–	3.9	4.5	3.0	4.0	
10	9193	3.8	5.1	4.4	–	–	4.3	4.5	3.6	4.3	5.6
	9209	6.9	5.6	8.4	–	–	6.7	7.4	6.6	6.9	
17.5	9208	11.3	8.8	Dead thirty-second day						10.0	10.0
	9211	9.3	10.7	Off drug thirty-second day, dead thirty-ninth day						10.0	
25	9202	25.0	35.6	Dead twenty-ninth day						30.3	21.7
	9206	13.2		Off drug tenth day, sacrificed fifty-third day						13.2	

* Each concentration within a two-week period is the average of 2 to 6 determinations.
– = no determination

DISCUSSION

Isonicotinic acid hydrazide (Nydrazid) was found to be rapidly and completely absorbed from the gastrointestinal tract of mice and dogs. In mice there was a very small difference between the oral, subcutaneous, and intravenous lethal doses. The early peak plasma drug concentrations obtained with oral administration in dogs were essentially the same as those achieved by intravenous administration. These plasma concentrations declined not more than 66 per cent in five hours and disappeared almost completely in sixteen hours. No cumulation of plasma concentrations was observed on an eight-hour, twice-daily dose schedule in dogs at or below 17.5 mg. per kg. per day.

The observed time lag between attainment of peak plasma concentrations and onset of convulsive seizures suggests either a delay in passage of the drug across the blood-brain barrier or conversion to a toxic metabolic intermediate,

or both. Good correlation was obtained between plasma drug concentration and toxicologic effects. Plasma levels of approximately 44 γ per ml. were obtained in mice receiving single LD_5 oral dosage. In chronic dog studies, plasma drug concentrations of 9 to 35 γ per ml. were associated with anorexia, central nervous system stimulation, liver damage, and death within one month; concentrations of 6 to 8 γ per ml. were not lethal in sixteen weeks but caused only anorexia and transient central nervous system stimulation; lower concentrations caused little or no anorexia and no central nervous disturbances in the same period of study.

The pathologic and biochemical evidence indicates that the jaundice produced at the higher dose levels in dogs is the result of fatty degeneration of the liver. The slight renal tubular damage found may have been secondary to the hepatic insufficiency. When drug administration was stopped in one moribund jaundiced dog on the thirty-third day, no significant liver damage, but considerable regeneration of hepatic parenchyma, was found when the dog died six days later. Another jaundiced dog was taken off the drug after ten days, and at autopsy forty-three days later the liver showed very few localized areas of fatty degeneration (table 2). In the latter dog, bilirubin tolerance returned to normal and signs of central nervous system disturbance disappeared. Thus, it appears that the two most outstanding manifestations of isonicotinic acid hydrazide toxicity may be reversible.

In mice, rats, and dogs, the experimental maximum tolerated subacute or chronic oral doses agreed closely with those calculated on a basis of relative body surface area. In the order of species named, these doses were approximately 64, 40, and 10 mg. per kg. per day. Extension of these calculations to man on a similar basis indicates that the maximum tolerated chronic oral dose may be as high as 4 to 5 mg. per kg. per day for comparable periods of time. At present, dosage approaching this level is undergoing clinical trial (5).

SUMMARY

Isonicotinic acid hydrazide (Nydrazid) was found to be rapidly absorbed after oral administration to mice and dogs. At the acute and chronic dosages employed in dogs in this study, approximately sixteen hours were required for disappearance of the drug from circulating plasma.

Acute toxicity in mice and dogs was characterized by excitement and convulsions which were delayed in onset even after intravenous administration. Subacute toxicity in rats was evidenced by anorexia, loss in body weight, occasional convulsions, and slight hepatic damage.

Chronic administration to dogs resulted in anorexia, loss in body weight, ataxia, tonic and clonic convulsions, fatty degeneration of the liver, and jaundice. Reversibility of these effects appears possible upon prompt cessation of drug administration.

The subacute and chronic maximum tolerated daily oral dose of isonicotinic acid hydrazide in different species appears to be related to their relative body surface areas.

SUMARIO

Farmacología de la Hidracida del Acido Isonicotínico (Nydrazid)

La hidracida del ácido isonicotínico (*Nydrazid*) fué, según se observó, rápidamente absorbida después de ser administrada oralmente a los ratones y perros. A las dosis agudas y crónicas empleadas en los perros en este estudio, necesitáronse unas dieciséis horas para la desaparición de la droga del plasma circulante.

La intoxicación aguda por la droga se caracterizó en los ratones y perros por excitación y convulsiones, tardías en su iniciación aun después de la administración endovenosa. La intoxicación subaguda se tradujo en las ratas por anorexia, pérdida de peso, convulsiones ocasionales y leves lesiones hepáticas.

La administración crónica a los perros dió por resultado anorexia, pérdida de peso, convulsiones tónicas y clónicas, degeneración grasa del hígado e ictericia. Parece posible obtener la reversibilidad de esos efectos si se suspende prontamente la administración de la droga.

La máxima dosis oral tolerable, tanto aguda como crónica, de hidracida del ácido isonicotínico en diversas especies parece enlazarse con las relativas áreas superficiales del cuerpo de las mismas.

RESUME

Pharmacologie de l'hydrazide de l'acide isonicotinique (Nydrazid)

Chez la souris et le chien, il a été observé qu'après l'administration orale, l'hydrazide de l'acide nicotinique était rapidement absorbée.

Au cours de cette étude chez le chien, avec les doses utilisées pour une période d'administration brève, ou avec celles utilisées pour une période d'administration prolongée, il a fallu environ seize heures pour que ce composé disparaisse du plasma circulant.

Chez la souris et le chien, les phénomènes de toxicité aiguë étaient caractérisés par de l'excitation et des convulsions d'apparition tardive, même après l'administration intraveineuse. Chez le rat, les phénomènes de toxicité subaiguë se sont manifestés sous la forme d'anorexie, de perte de poids, de convulsions intermittentes et d'une légère altération du tissus hépatique.

L'administration prolongée, chez le chien, a provoqué l'anorexie, la perte de poids, l'ataxie, les convulsions toniques et cloniques, la dégénérescence graisseuse du foie et l'ictère. La réversibilité de ces effets apparaît possible si on cesse brusquement l'administration du produit.

En ce qui concerne la tolérance chez les différentes espèces, le maximum de la dose orale quotidienne de l'hydrazide de l'acide isonicotinique, cette tolérance paraît en rapport avec le poids de l'animal.

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HYDRAZINE DERIVATIVES OF ISONICOTINIC ACID (RIMIFON[1], MARSILID[2]) IN THE TREATMENT OF ACTIVE PROGRESSIVE CASEOUS-PNEUMONIC TUBERCULOSIS[3]

A Preliminary Report

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(Received for publication January 19, 1952)

INTRODUCTION

In the synthesis of the antituberculous compound thiosemicarbazone of isonicotinaldehyde (1), Dr. H. H. Fox of the Roche Chemical Research Laboratories used isonicotinic acid hydrazide as an intermediate (2). Dr. R. J Schnitzer and Dr. E. Grunberg in the Roche Chemotherapy Laboratories found that this compound exerts a striking and lasting effect on experimental tuberculosis in mice. Dr. Fox thereupon undertook the synthesis of a series of derivatives of isonicotinic acid hydrazide (3), which were then studied in Dr. Schnitzer's laboratory. The finding that some of these exerted antituberculous activity similar to the original isonicotinic acid hydrazide (4, 5), coupled with experimental suggestion of sufficient overlap of therapeutic and nontoxic dosage ranges (6, 7, 8), has led to the current clinical study. Three compounds have been consecutively and concurrently investigated by the present writers: (*1*) 1-isonicotinyl-2-glucosyl hydrazine[4], (*2*) 1-isonicotinyl-2-isopropyl hydrazine (Marsilid) and (*3*) isonicotinic acid hydrazide (Rimifon).

The studies, which are still in progress, now cover a six-month period. The compounds under investigation have been found to exert an important therapeutic effect in human tuberculosis (9). A preliminary report of the current status of these investigations is therefore advisable, although, by its very nature, many details must be omitted or briefly mentioned, and conclusions are necessarily tentative.

The initial therapeutic series in the present study comprises 97 consecutive patients with pulmonary tuberculosis who have now had four to fifteen weeks of therapy and were variously selected to allow study of many aspects of possible chemotherapeutic response. Within this series, a group of 44 consecutive cases was specially selected for the study of chemotherapeutic effect on acute active progressive bilateral caseous-pneumonic tuberculosis. It is this latter group which is described in the present report in an evaluation of the therapeutic

[1] Proprietary name, Hoffmann-La Roche, Inc., formerly designated as Ro 2-4572/1. This drug was supplied by the Scientific Laboratories of Hoffmann-La Roche, Inc., of Nutley, New Jersey.

[2] Proprietary name, Hoffmann-La Roche, Inc., formerly designated as Ro 2-3973. This drug was supplied by the Scientific Laboratories of Hoffmann-La Roche, Inc., of Nutley, New Jersey.

[3] From the Medical Service of Sea View Hospital, Staten Island, New York.

[4] Formerly designated Ro 2-4179. This drug was supplied by the Scientific Laboratories of Hoffman-La Roche, Inc., of Nutley, New Jersey.

effect of the hydrazine derivatives of isonicotinic acid in this important and serious type of pulmonary tuberculosis.

PLAN OF INVESTIGATION

Choice of Material

The fundamental criterion which was applied in the selection of each patient was that his disease had progressed so far that he was no longer a candidate for any other standard accepted form of therapy. No patient was chosen for whom

TABLE 1

SPUTUM EXAMINATIONS FOR ACID-FAST BACILLI BEFORE ONSET OF THERAPY

Total Patients: 44

	Number of Patients
Positive on unconcentrated smear: Gaffky VI–X	27
Positive on unconcentrated smear: Gaffky I–V	15
Positive on concentrated or gastric specimens	2
	44

TABLE 2

TEMPERATURE RANGE BEFORE ONSET OF THERAPY: ONE TO FIVE MONTHS

	Number of Patients
Range 100°F.	16
Range 101° to 102°F.	18
Range 103° to 105°F.	10
	44

TABLE 3

WEIGHT LOSS (POUNDS) DURING PRE-THERAPY COURSE

Weight Loss from Normal Weight	*Number of Patients*
1–9 pounds	4
10–19 pounds	13
20–29 pounds	9
30–39 pounds	10
40–49 pounds	5
50–59 pounds	3
26½ pounds average weight loss	44

benefit from bed rest, the currently available antimicrobial therapy, medical collapse, or surgical therapy could be anticipated. Because of the rigidity of this criterion, it is evident that only patients with extensive disease were selected, the so-called "hopeless" case, in many instances close to terminal status. A sufficient pre-therapy observation period was utilized to preclude the selection of any patient who was improving either spontaneously or with other therapy. Each

patient in this study, during an average pre-therapy observation period on the wards of 5.2 months per patient, was observed to have either stationary or progressive disease. In each instance the prognosis was determined to be uniformly poor. In all instances the sputum was persistently positive for *M. tuberculosis* (table 1); and, in all, the temperature was continuously elevated from 100° F.

TABLE 4

PREVIOUS THERAPY: 44 CASES

1. Streptomycin		41 patients
One course—17 patients	Average:	50 gm.
Two courses—14 patients	Average:	84 gm.
Three courses—9 patients	Average:	142 gm.
Five courses—1 patient	Average:	100 gm.
No previous streptomycin therapy		3 patients
2. Para-aminosalicylic acid therapy*		39 patients
Average duration of therapy		3 months
3. Pneumothorax therapy		13 patients
4. Pneumoperitoneum therapy		9 patients
5. Surgical therapy (excisional, et cetera)		4 patients

* Administered concurrently with streptomycin

TABLE 5

CLASSIFICATION OF PULMONARY DISEASE: 44 CASES

(Classification of Ornstein *et al.* (10))

	Number of Cases
Exudative-caseous-pneumonic	21
Productive-caseous-pneumonic	7
Caseous-pneumonic	8
Exudative-productive-caseous-pneumonic	8
	44

TABLE 6

CLASSIFICATION OF PULMONARY DISEASE: 44 CASES

(National Tuberculosis Association Classification)

	Number of Cases
Minimal	0
Moderately advanced	5
Far advanced	39
	44

to 105° F. (table 2) for periods of one to five months. All patients had exhibited weight loss (table 3) and had failed to gain appreciably for protracted periods despite other forms of therapy (table 4).

The classification of the tuberculosis present in the 44 patients may be seen in tables 5, 6, and 7. The patients are further classified by race, sex, age, and duration of acute disease in table 8.

TABLE 7

ROENTGENOGRAPHIC ANALYSIS OF PRE-THERAPY COURSE

	Number of Cases
Progressive	31
Recent spreads of disease	6
Stationary	13
	44

TABLE 8

DATA CONCERNING THE 44 PATIENTS AT ONSET OF THERAPY

NUMBER OF PATIENTS										DURATION OF DISEASE		DURATION OF PRESENT ILLNESS	
Age					Race			Sex					
10–19	20–29	30–39	40–49	50–59	White	Negro	Puerto Rican	Male	Female	Range	Average	Range	Average
										months	*months*	*months*	*months*
3	15	12	8	6	29	8	7	17	27	2 to 276	30	1 to 46	14

TABLE 9

CLINICAL STATUS AT ONSET OF THERAPY

	Number of Patients
Appetite	
Good	4
Fair	7
Poor	26
Bad	7
	44
Energy and Sense of Well-Being	
Good	2
Fair	7
Poor	29
Bad	6
	44
Toxicity	
one plus	10
two plus	17
three plus	10
four plus	7
	44
Cough and Sputum	
0	1
one plus	6
two plus	15
three plus	16
four plus	6
	44

Patient Status Before Therapy

Clinical symptomatology uniformly present among these patients included excessive cough and expectoration and anorexia and asthenia, graded independently by the patient and the medical and nursing staffs. General estimates of toxicity were more difficult to obtain but have been roughly graded (table 9). Five patients also exhibited other forms of tuberculosis, including laryngeal tuberculosis, otitic tuberculosis, intestinal tuberculosis, and tuberculous empyema. Nontuberculous diseases included asthma, sickle-cell anemia, previous psychosis, alcoholism, recurrent urticaria, probable amyloidosis, and syphilis.

Methods

Before institution of therapy, each patient was subjected to clinical and laboratory study. The studies included physical examination and, in cases of doubt, consultation with members of the consulting services. Patients had preliminary chest roentgenograms, complete blood counts, urinalyses, chemical studies of the blood, including determination of cephalin flocculation, cholesterol and cholesterol esters, serum proteins, icterus indices, and van den Bergh tests. Finally, each patient was evaluated from the psychologic standpoint with a view to ensuring perseverance, cooperation, and emotional stability. No patient with overt evidence of serious parenchymatous disease (other than pulmonary tuberculosis) was accepted for therapy during the early phases of the study.

Dosage Regimens

The initial trials with 1-isonicotinyl-2-glucosyl hydrazine (figure 1) during June, July, and August and, later in September, 1951 (11) demonstrated convincingly that humans readily tolerate 2 mg. of this compound per kg. of body weight. Since animal studies with isonicotinic acid hydrazide (Rimifon, figure 2) and its isopropyl derivative (Marsilid, figure 3) indicated that greater therapeutic benefit could be anticipated from these more stable compounds, their study was started using an initial dose approximating 2 mg. per kg. of body weight. Each drug was administered by mouth after meals in three divided daily doses. All other medication, including vitamins, and all other treatments, including pneumoperitoneum, were discontinued at onset of therapy. Three therapeutic pneumothoraces were continued in instances wherein control of the tuberculosis had been established in one lung but the contralateral lung remained uncontrolled.

Patients were introduced into the series in groups. The first group, 17 patients, started therapy with the isopropyl derivative (Marsilid) on October 2, 1951. Additional groups at 2 mg. per kg. were added on October 15 (6 patients) and October 25 (7 patients). On November 15, after more than seven weeks of observation and experience at the 2 mg. dosage level, a new 29-patient study was started, using 4 mg. per kg. of body weight. On November 20, the entire original series consisting of three groups (30 patients) was elevated to the 4 mg. dosage level and, on November 23, 17 additional patients were added at the same level. Absence of significant toxicity (see below) appeared to justify sharp addi-

tional increases in dosage and, on December 6, 10 additional patients were started on therapy at 10 mg. per kg. The decision to utilize this dosage level appears to have been justified. On December 17, 11 patients began therapy with isonicotinic acid hydrazide (Rimifon) at a dosage of 2 mg. per kg. After one week, this dosage was elevated to 4 mg. per kg. Of the 97 patients in the above therapeutic groups, 44 suffered from active progressive caseous-pneumonic tuberculosis with fever; they form the basis for this report.

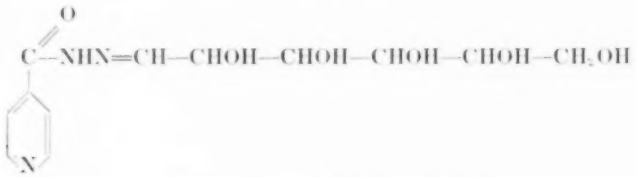


FIG. 1. 1-Isonicotinyl-2-glucosyl hydrazine

FIG. 2. Isonicotinic acid hydrazide
FIG. 3. 1-Isonicotinyl-2-isopropyl hydrazine

RESULTS

Tuberculosis is both a systemic and a localized disease. The systemic features are probably responsible for the toxic manifestations, notably asthenia, temperature elevation, weight loss, and anorexia. The localized lesion is best exemplified by the cavity and manifests itself through the sputum and the roentgenogram. The acute caseous-pneumonic tuberculous lung lesions combine both of these and therefore offer a vigorous challenge to any therapeutic agent or modality. This report is concerned chiefly with the results of therapy with the hydrazide derivatives of isonicotinic acid in this type of disease. In order, then, to properly evaluate their effectiveness, the results will be recorded under two main headings. The first to be considered will be the systemic response.

Systemic Response

Temperature: All patients were febrile for varying periods at the onset of therapy (table 2). In 42 of the 44 patients under treatment, the temperatures subsided promptly and sometimes precipitously. In some patients the temperature returned to normal in as few as two days; in others, twenty-one days were required. Almost all temperatures were normal by the tenth day of therapy.

Defervescence has been sustained in all cases after the initial fall with the exception of 6 instances of upper respiratory infection. In these patients, a temperature elevation to approximately 100° F. occurred for three to four days, after which the temperature returned to normal.

TABLE 10

AVERAGE WEIGHT GAIN AFTER THERAPY

(Duration of Therapy: Four to Fifteen Weeks*)

	Number of Patients
Total Patients—44:	
Weight gain 0 to 9 pounds	11
Weight gain 10 to 19 pounds	11
Weight gain 20 to 29 pounds	11
Weight gain 30 to 39 pounds	8
Weight gain 40 to 49 pounds	2
Weight gain 50 to 59 pounds	1
	44
Male Patients: (17) Average gain	16.5 pounds
Female Patients: (27) Average gain	22.0 pounds

* See table 11

TABLE 11

WEIGHT GAIN IN RELATION TO DURATION OF THERAPY AND DOSAGE RANGE

GROUPS	NUMBER OF CASES	DOSAGE RANGE	WEEKS OF TREATMENT	AVERAGE WEIGHT GAIN
				pounds
1	5	2 to 4 mg. Marsilid*	15	22.8
2	1	2 to 4 mg. Marsilid	13	32.0
3	6	2 to 4 mg. Marsilid	12	26.3
4	9	4 mg. Marsilid	9	27.5
5	10	4 mg. Marsilid	8	22.0
6	7	10 mg. Marsilid	6	7.9
7	6	2 to 4 mg. Rimifon†	4	7.0

Average duration of therapy = 8.8 weeks
Average weight gain = 19.7 pounds

* 1-Isonicotinyl-2-isopropyl hydrazine
† Isonicotinic acid hydrazide

Weight: The weight gains have been most spectacular (table 10). Forty-four patients have gained an average of 19.7 pounds each in the average of 8.8 weeks. The gains have usually become evident in the second or third weeks and are best appreciated by analysis of the series (table 11). The average weight gain among 17 males was 16.5 pounds; among 27 females, 22.0 pounds. It is essential to note that, although diligently sought, abnormal accumulations of body fluids were not found. Indeed, one of the patients with edema due to probable amyloidosis

experienced a sudden and profound diuresis and loss of edema. It should be noted that almost every patient who has been on therapy for eight weeks or longer is now back to normal weight. Thus, of 31 patients who have had eight to fifteen weeks of therapy, there has been an *average* weight gain of 24.9 pounds.

Appetite: Appetites are not susceptible of objective estimation but have been uniformly remarkable in the present series. The best descriptive stock expression is "ravenous." Based on the calculations of the dietary division, there has been at least a 50 per cent increase in food consumption on the wards utilized for this study.

Energy and sense of well-being: These features are similarly difficult to evaluate, and it is a matter of major perception to eliminate the psychologic aspects inherent in a group entirely aware that it is receiving special attention. Nevertheless, the improvement in these two spheres has been strikingly obvious to the nursing and medical staffs and to the patients. Indeed, disciplinary problems have been unusually common, a factor which is obviously attributable to resurgent animal vigor. Apathy and drowsiness, characteristically seen among many of the acutely ill tuberculous patients on the wards, have been completely eliminated by the second week in all instances. The routine inquiries after institution of therapy indicate that the patients usually feel better before any objective measurements of improvement can be made.

Skin: When absent, there was restoration of normal skin oiliness and turgor by the second or third weeks. It has become evident that changes in skin appearance often presage subsequent clinical improvement.

Response of Local Lesion

Cough and expectoration: Relief from cough and expectoration was noted with regularity during the second week of therapy. In most instances, loss of expectoration preceded loss of cough. This is probably a reflection of the fact that, among other things, cough in tuberculous patients arises from increased fluid material within the bronchial tree and from direct irritation of the bronchial mucosa. It may be assumed, therefore, that diminution in expectoration preceded resolution of the bronchial irritation.

Cough preceding institution of therapy has been arbitrarily graded in plusses. Reference to table 12 will demonstrate the sharp diminution in cough experienced by patients under therapy. After the second week, no patient requested antitussives and none are in use on the wards at this time.

Expectoration is roughly measurable. The sputum cup in use at Sea View Hospital is a 240 cc. receptacle. The amounts of sputum raised have been graded as follows: none to one-half cup, *1 plus;* one-half to one cup, *2 plus;* one to two cups, *3 plus;* three or more cups, *4 plus.* In table 12 the marked reductions encountered are graphically portrayed.

Sputum: Unconcentrated specimens of sputum were examined once weekly by the stained smear technique. It has been the custom at Sea View Hospital to supplement these with concentrated specimens after two consecutive smears have been negative for acid-fast bacilli; and with concentrates of the gastric

contents when concentrates of sputum are negative or when there is insufficient expectorated material for analysis. This scheme has been followed in the present study. Obviously, reference to the dates on which therapy was started will indicate the reasons for omission of cultures and the transitory status of the findings at this writing. No attempt at detailed analysis would be valid in this preliminary statement. The figures are presented in table 13.

TABLE 12

EFFECT OF THERAPY (FOUR TO FIFTEEN WEEKS*) ON COUGH AND SPUTUM

	BEFORE TREATMENT	ON TREATMENT
	Number of patients	*Number of patients*
0	1	24
1 plus	6	15
2 plus	15	4
3 plus	16	1
4 plus	6	0
Total	44	44

* See table 11

TABLE 13

EFFECTS OF THERAPY (FOUR TO FIFTEEN WEEKS*) ON PRESENCE OF ACID-FAST BACILLI IN SPUTUM

	BEFORE TREATMENT	ON CURRENT TREATMENT
	Number of patients	*Number of patients*
Positive on unconcentrated smear:		
Gaffky VI–X	27	6
Positive on unconcentrated smear:		
Gaffky I–V	15	19
Alternating positive and negative	2	11
Negative (including gastric examination)	0	8
Total	44	44

* See table 11

Since the introduction of antimicrobial therapy into the treatment of tuberculosis, open cavities in association with sputum negative for tubercle bacilli have been noted with increasing regularity. It is not surprising then that this phenomenon was observed in 8 cases in the present series. It should be noted that 41 of the 44 patients had previously received streptomycin without "conversion"; 39 of these received *p*-aminosalicylic acid (PAS).

It is specious to quantitate sputum bacillary counts in smear and concentrate without consideration for the quantities of sputum raised. Thirty-two ounces of Gaffky X sputum reflect one underlying pathologic state; one ounce of Gaffky I reflects another. The degree of reversibility of the pathology will be the subject of later estimation.

Roentgenograms: Chest films were taken at onset of therapy and monthly thereafter. Tomographs and special exposures were made as required. The current evaluation represents a three-month observation on the older cases. In some instances, as indicated, it represents a one-month follow-up. Reference is made to the initial classification presented in tables 5 and 6, and an estimate of the current status may be seen in table 14.

Realizing that roentgenographic changes would be last in making their appearance, the present investigators have not been surprised by the findings presented in table 14. In three of the instances recorded as "cavities smaller," the standard film suggests complete closure. Since tomograms have not been taken to date, the exact determination will have to be held in abeyance.

TABLE 14

EFFECT OF THERAPY (FOUR TO FIFTEEN WEEKS) ON ROENTGENOGRAPHIC APPEARANCE OF LESIONS

GROUP	NUMBER OF PATIENTS	WEEKS OF TREATMENT	EXUDATION DIMINISHED	CAVITIES SMALLER	NO CHANGE
1	5	15	0	0	5
2	1	13	0	1	0
3	6	12	0	5	1
4	9	9	3	4	2
5	10	8	2	4	4
6	7	6	0	3	4
7	6	4	0	0	6
Total	44		5	17	22

Toxicity

Evaluation of "chronic" toxicity is without rationale in this report. "Acute" toxicity deserves close scrutiny and the salient features of the untoward reactions encountered may be seen in table 15.

Twitching of the extremities and increased deep reflexes were the most frequently encountered symptoms. Constipation and vertigo followed in frequency. Detailed analyses, presented elsewhere (12), show that symptoms occurred most commonly in the second week, remained fairly constant into the fourth week, and declined sharply thereafter. With the isopropyl derivative (Marsilid) an increasing frequency of symptomatology roughly parallels increasing dosage. Isonicotinic acid hydrazide (Rimifon) produced fewer side reactions per patient; its reported "chronic" toxicity in animals (6) dictates caution in the use of higher doses.

Laboratory Findings

The preliminary investigations of June and July, 1951, were undertaken under most rigid controls. At onset, daily urine examinations and thrice-weekly blood counts were performed. These were supplemented by weekly cephalin flocculation tests, van den Bergh tests, and erythrocyte sedimentation rates. Other

tests were performed as indicated. Emboldened by the absence of positive findings, the laboratory observations were performed less frequently in the later groups. It was considered advisable to obtain urine examinations, complete blood counts, erythrocyte sedimentation rates, and cephalin flocculation tests at weekly intervals during the early weeks of treatment. After one month of therapy, these

TABLE 15

TOXICITY AND SIDE EFFECTS WITH ISONICOTINIC ACID HYDRAZIDE (RIMIFON) AND 1-ISONICOTINYL-2-ISOPROPYL HYDRAZINE (MARSILID) THERAPY

	NUMBER OF PATIENTS NOTING SYMPTOMS	DIZZINESS	CONSTIPATION	TWITCHING EXTREMITIES	INSOMNIA	DROWSINESS	HEADACHE	LEG WEAKNESS	MYOPIA	TINNITUS	HYPERREFLEXIA	DELAYED STREAM	MOUTH DRYNESS	DYSPNEA	NUMBER OF TIMES SYMPTOMS NOTED
Group I. 12 patients 1-isonicotinyl-2-isopropyl hydrazine (Marsilid)															
2 mg/kg 10/2/51 to 11/20/51	7	2	6	1										2	11
4 mg/kg 11/20/51 to 1/15/52	10	7	2	9	4	3		2	1	1	5	2	1	3	40
Group II. 19 patients 1-isonicotinyl-2-isopropyl hydrazine (Marsilid)															
4 mg/kg 11/15/51 to 1/15/52	17	11	13	15	9	13	1	11	4	1	11	7	4	1	104
Group III. 7 patients 1-isonicotinyl-2-isopropyl hydrazine (Marsilid)															
10 mg/kg 12/5/51 to 1/15/52	7	5	6	7	4	3	2	6	1	2	6	4	3	2	51
Group IV. 6 patients isonicotinic acid hydrazide (Rimifon)															
2 mg/kg 12/17/51 to 12/24/51	3		1			1		1							3
4 mg/kg 12/24/51 to 1/15/52	6	1	1	5			1				1		1		10

observations were made at fortnightly intervals; and after two months of therapy, the determinations were made at three-week or longer intervals.

Complete detailed analysis is not within the scope of the present report. A summary of the results, however, indicates that:

Hemoglobin and erythrocytes are virtually unaffected. Losses up to 0.5 gm. per 100 cc. of hemoglobin are noted in a few isolated instances by the third and fourth weeks. Restoration occurs promptly, spontaneously, and without specific therapy.

The leukocytes continue unchanged.

The erythrocyte sedimentation rates are essentially unaffected; in some instances a tendency toward minor elevation is noted.

Cephalin flocculation tests reveal no evidence of liver damage.

The findings in the urine were not remarkable. An occasional trace of albumin was noted; investigation failed to reveal renal changes in any instance. A single exception to this statement is described in the case report below of a patient (K. R., Case 3) with probable amyloid nephrosis and persistent proteinuria before and during therapy.

No changes were noted in electrocardiograms, determinations of serum proteins, icterus indices, van den Bergh's reactions, or serum cholesterols.

Representative Case Reports

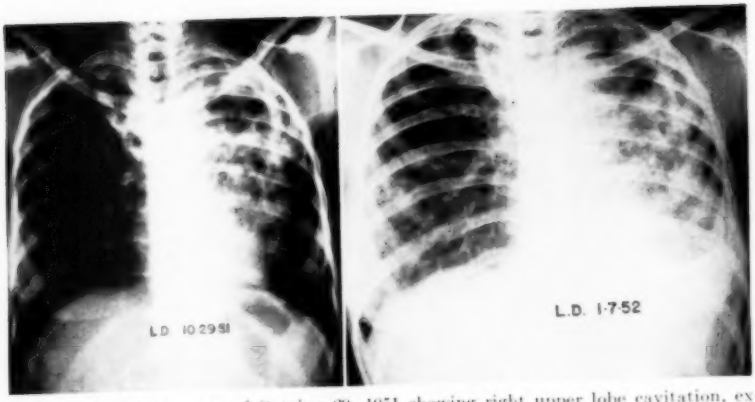
Case 1 (L. D.). This 32-year-old Puerto Rican female was admitted to Sea View Hospital on June 16, 1951 with extensive bilateral caseous-pneumonic tuberculosis. She had been ill since 1941. In the two-year period 1950 through 1951 she had had six courses of streptomycin totaling 306 gm., thirteen months of pneumothorax without arrest of the disease, and ten months of pneumoperitoneum which was similarly ineffective. Thirteen months of PAS therapy, with and without streptomycin, did not reverse the steadily progressive course of her disease.

Before the onset of therapy with the isopropyl derivative (Marsilid), the patient was markedly toxic, weak, unable to leave her bed, and weighed 84 pounds, as compared with her normal weight of more than 100 pounds. She had a hacking, almost constant cough, which was productive of approximately 480 cc. (i.e., two cupfuls) of purulent sputum a day. The sputum was rich in tubercle bacilli on all examinations. The patient was febrile, with a temperature which ranged from 103° to 104° F. for the previous four months. Amenorrhea had been present for ten months. Roentgenographic examination showed extensive bilateral cavitary, caseous-pneumonic disease (figure 4).

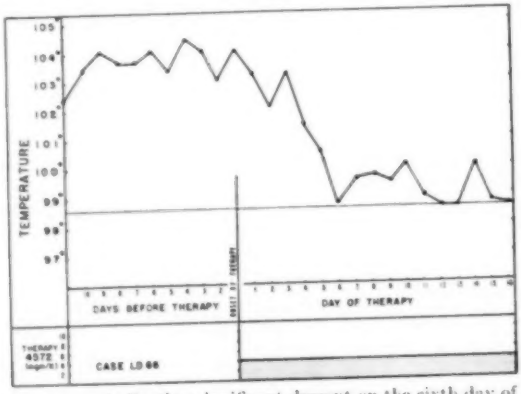
Therapy with the isopropyl hydrazine derivative (Marsilid) was begun on November 23, 1951, at 4 mg. per kg. per day. Within one week there was a clinical metamorphosis. A sharp reversal of her highly toxic and almost moribund condition took place. An excellent appetite was noted on the fourth day of therapy, her fever reached almost normal range on the sixth day of therapy, and in ten days her cough was markedly reduced and was productive of only 90 cc. of sputum. The improvement continued, and in another week cough and sputum were practically gone. She remained afebrile after the tenth day of therapy, and on the twenty-fourth day of treatment was able to leave her bed for the first time in more than a year. Her strength rapidly returned, no evidence of toxicity remained, and barely enough sputum for bacteriologic examination was expectorated. After six weeks of therapy her menses reappeared. At the third week of therapy, her weight was 82 pounds and by the eighth week it was 97 pounds.

A roentgenogram on January 7, 1952 showed some improvement, with cavities somewhat smaller but present. The sputum remains positive for *M. tuberculosis* but is now persistently in a low Gaffky range. Patient is now clinically well, without cough or expectoration, has an excellent appetite, and is continuing therapy with the isopropyl derivative (figure 4).

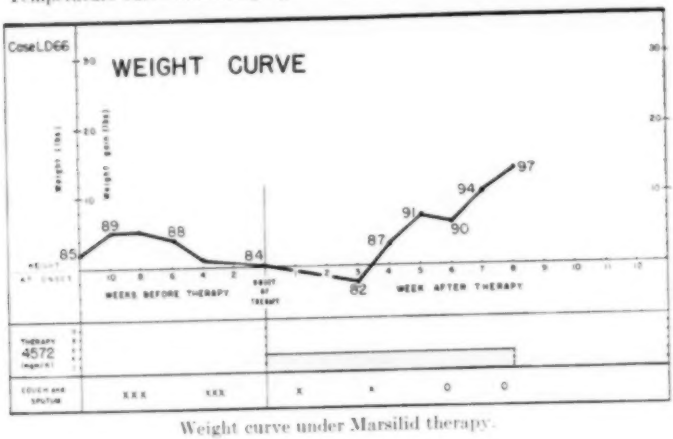
Case 2 (J. W.). This 34-year-old Negro male was admitted to Sea View Hospital on July 17, 1951. Pulmonary symptoms first appeared in August 1950, when he was treated with a 49 gm. seven-week course of streptomycin. This did not alter the progress of his disease and he was admitted to Sea View with far advanced, progressive, bilateral caseous-pneumonic tuberculosis. The disease was complicated by severe tuberculous laryngitis. During May and June of 1951 he received a second course of 42 gm. of streptomycin without sig-



(Left). Roentgenogram of October 29, 1951 showing right upper lobe cavitation, extensive destruction of the left lung, and a small residual pneumothorax.
(Right). Roentgenogram of January 7, 1952 showing diminution in cavitation bilaterally.



Temperature curve indicating significant descent on the sixth day of therapy



Weight curve under Marsilid therapy.

FIG. 4. *Case 1* (L. D.)

nificant effect. A third 42 gm. seven-week course was given in September and October, 1951. He had simultaneously had nine months of PAS therapy.

This streptomycin-PAS therapy, combined with strict bed rest, failed to halt the patient's downhill course. He remained toxic, weak, and moderately febrile with temperatures ranging from 101° to 102°F. throughout the entire hospital course. He slowly lost weight until he was 40 pounds below normal. He had a severe cough, productive of approximately 480 cc. of purulent sputum daily which was persistently positive for acid-fast bacilli in high Gaffky range. His tuberculous laryngitis was characterized by severe hoarseness and pain on deglutition.

Therapy with the isopropyl hydrazine derivative (Marsilid) was begun on October 26, 1951, at 2 mg. per kg. per day. The patient's temperature reached normal within one week and his cough was sharply reduced. There was clear-cut reduction in toxicity. On November 20, 1951, the daily dosage was increased to 4 mg. per kg. He continued to improve and six weeks after institution of therapy had practically no cough or expectoration. His laryngeal symptoms had completely disappeared, he was afebrile and rapidly gaining weight. His appetite was excellent.

The sputum has remained positive for acid-fast bacilli although now is in the low Gaffky range. The patient remains clinically well and therapy is being continued.

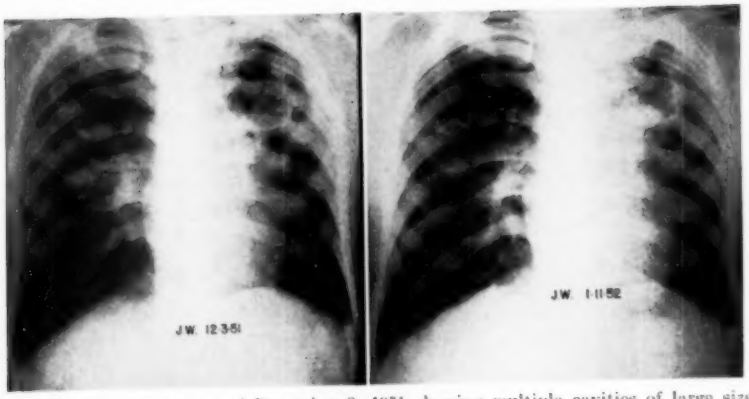
Comparison of interval with pre-therapy film shows marked reduction in cavity size; this is seen in *each* of the cavities present. Although spontaneous closure of single cavities is not unusual, it is uncommon for many cavities to close simultaneously (figure 5).

Case 3 (K. R.). This 24-year-old Negro female was admitted to Sea View Hospital on November 7, 1951. Although symptoms of her pulmonary tuberculosis were first noted in 1948, there was marked exacerbation in October 1951. On admission to Sea View Hospital on November 7, 1951, her status was critical. In the previous months her weight had decreased from a normal of 125 pounds to approximately 80 pounds. She was highly toxic and had been amenorrheic since early in 1951. Marked anorexia, lethargy, and weakness, and moderately severe cough and expectoration were present. Her temperatures ranged from 103° to 104°F. There was persistent diarrhea, possibly due to tuberculous enteritis. Three plus pitting edema of the extremities and 2 plus albuminuria were present, possibly due to amyloidosis. Serologic tests for syphilis were positive. The sputum was highly positive for acid-fast bacilli on every examination.

Therapy with the isopropyl hydrazine derivative (Marsilid) was begun on November 24, 1951, at 4 mg. per kg. per day. Within a few days, the patient's temperature began to descend and reached normal in ten days. At the same time, there was a complete reversal in this patient's clinical state. Her appetite became ravenous. Diarrhea subsided completely within two weeks. The edema disappeared promptly during the first week of therapy. There was a remarkable gain in well-being. Four weeks after the onset of therapy, cough and expectoration had almost completely disappeared. Rapid weight gain was noted and after eight weeks of therapy approximately a 37-pound gain in weight was noted. (Accurate measurement of weight at the onset of therapy could not be made because the patient was too weak to stand on the scale.)

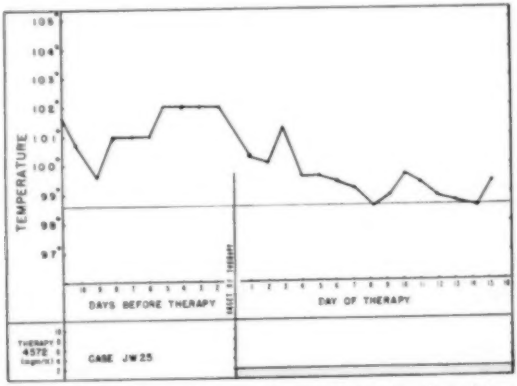
Two sputum examinations in the first two weeks of therapy were positive for acid-fast bacilli. However, the following week, smear of the sputum was negative for tubercle bacilli and five subsequent consecutive examinations have been negative, including a gastric aspiration on January 9, 1952.

A roentgenogram on January 7, 1952 showed marked improvement over the pre-therapy examination. The multiple cavities present in the left lung were all

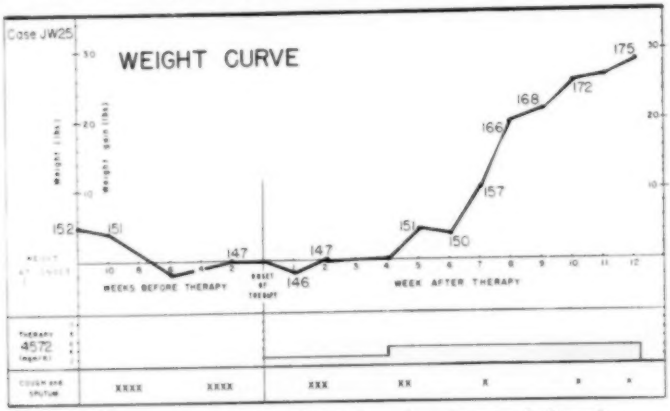


(Left). Roentgenogram of December 3, 1951 showing multiple cavities of large size throughout the upper two-thirds of the left lung. Exudative spread is seen on the right.

(Right). Roentgenogram of January 11, 1952 showing simultaneous reduction in sizes of several cavities.

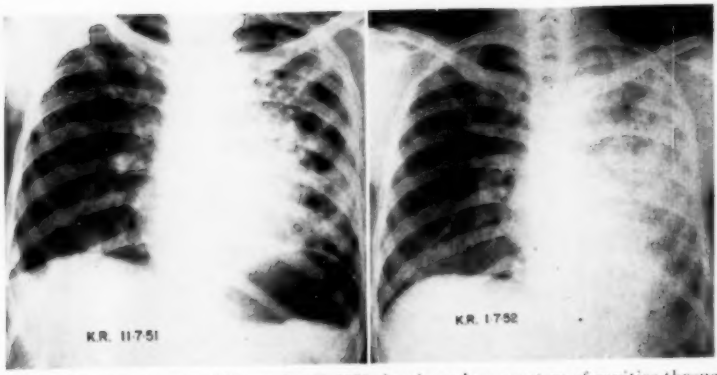


Temperature curve indicating continuous temperature elevation of 4 months' duration reached normal on the eighth day of Marsilid therapy.



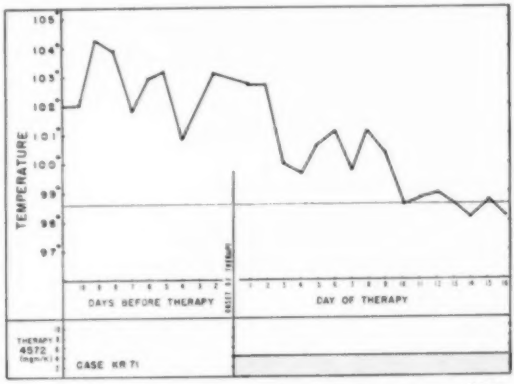
Weight curve showing significant alteration after the sixth week.

FIG. 5. *Case 2* (J. W.)

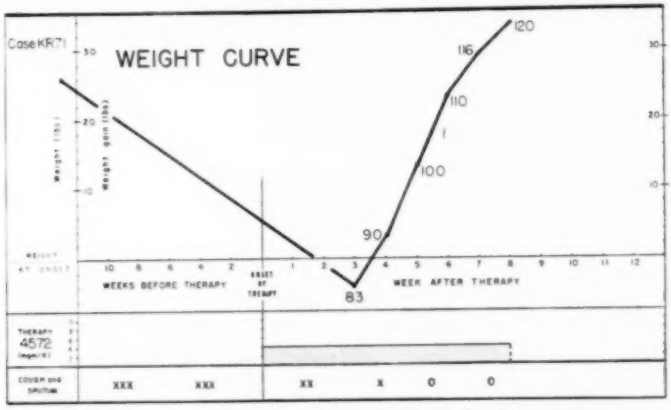


(Left). Roentgenogram of November 7, 1951 showing a large system of cavities throughout the left lung and an exudative lesion on the right.

(Right). Roentgenogram of January 7, 1952, showing cavities on the left markedly reduced, virtually closed.



Temperature curve indicating persistently elevated temperature which reached normal on the tenth day of therapy.



Weight curve indicating gain in the fourth week, rising sharply.

FIG. 6. *Case 3* (K. R.)

much smaller. The patient is continuing to receive therapy and is clinically completely asymptomatic, without cough or expectoration and with excellent appetite. She feels strong and is at normal weight (figure 6).

Case 4 (F. W.). This 29-year-old Negro female first suffered symptoms of pulmonary tuberculosis in October 1950. In June 1951, an exacerbation occurred and she was admitted to Sea View Hospital on November 1, 1951. She had previously received 60 gm. of streptomycin and five months of PAS therapy. On admission, she was classified as having far advanced, bilateral caseous-pneumonic tuberculosis. At the time of institution of therapy, she was highly toxic. There were marked anorexia, lethargy, five months' amenorrhea, and weight loss of 16 pounds in the previous eleven weeks. Fever ranged from 101° to 102°F. A moderately severe cough produced approximately 240 cc. of sputum a day.

Therapy with the isopropyl derivative (Marsilid) was begun on November 23, 1951 at 4 mg. per kg. per day. Within one day the patient's temperature became normal and it has remained so ever since. Marked concurrent clinical improvement was noted, with rapid reduction in cough and expectoration. Her symptoms of toxicity vanished, her appetite became excellent, and strength returned. Unable to leave her bed before therapy because of weakness, she resumed normal activity. Menstruation returned for the first time after one month of treatment.

Her sputum was positive for *M. tuberculosis* on every examination prior to onset of therapy, the bacilli being abundantly present on plain smear. On December 5, 1951 it was still positive for acid-fast bacilli on smear. Examination on December 12, 1951 was negative, however, and six subsequent examinations have similarly been negative for acid-fast bacilli, including an examination of gastric contents on January 14, 1952.

A roentgenogram on January 7, 1952 shows decrease in size of cavity with some resorption of the exudative disease present. She continues under therapy, is clinically asymptomatic, and without cough or expectoration. She has gained 20 pounds in eight weeks of therapy (figure 7).

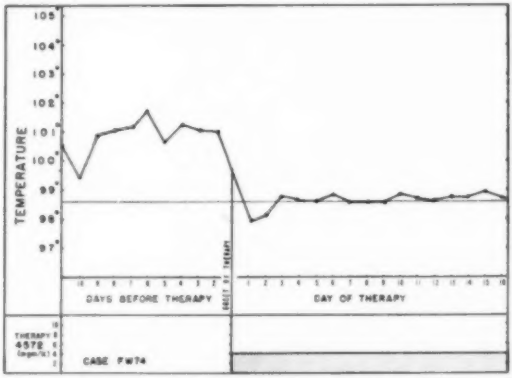
Case 5 (D. W.). This 15-year-old Negro girl was admitted to Sea View Hospital on May 29, 1951, shortly after the onset of symptoms of pulmonary tuberculosis. She was treated with three courses of streptomycin, totaling 100 gm., and PAS for one month. This therapy failed to alter her active, progressively downhill course, and in December her condition was critical. During the previous twelve weeks she had lost 7 pounds, reaching a low of 71 pounds. She was highly toxic, lethargic, anorexic, and amenorrheic. She had a moderate cough and expectoration which at each examination was highly positive for tubercle bacilli. She was continuously febrile since admission, with temperatures ranging between 101° and 103°F.

Therapy with the isopropyl derivative (Marsilid) was begun on February 5, 1951 at 10 mg. per kg. per day. Within a week her temperature reached normal levels, and cough and sputum were markedly decreased, so that she was hardly able to produce enough for bacteriologic examination. Concomitant disappearance of toxicity and a return of normal appetite occurred.

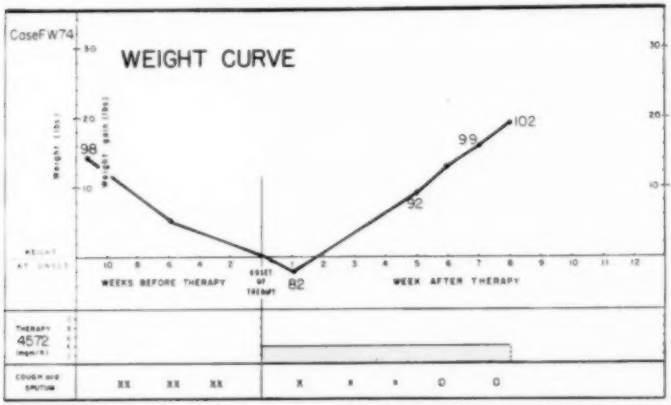
Clinical improvement has continued although the roentgenogram after one month of therapy shows no significant change. Clinically, she is completely asymptomatic and has gained 25 pounds in six weeks. She no longer has cough or expectoration, and remains afebrile. The first two sputum examinations after the onset of therapy were positive but her last examination on January 16, 1952 was negative for tubercle bacilli. Further observations, both clinical and bacteriologic, are being continued (figure 8).

(Left). Roentgenogram of November 5, 1951 showing large cavity in left upper lobe and exudate in lower lung field. Exudate with probable cavity in the first anterior interspace is noted on the right.

(Right). Roentgenogram of January 7, 1952 showing marked reduction and possible early closure of the cavities.



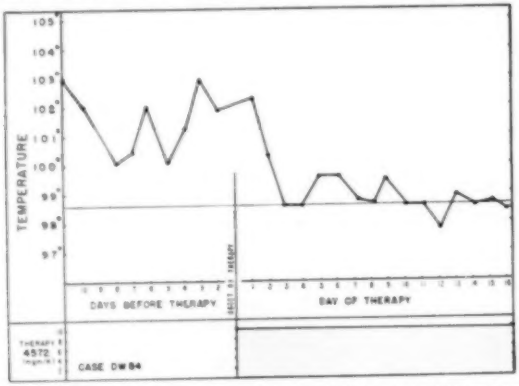
Temperature curve indicating prompt and persistent temperature subsidence.



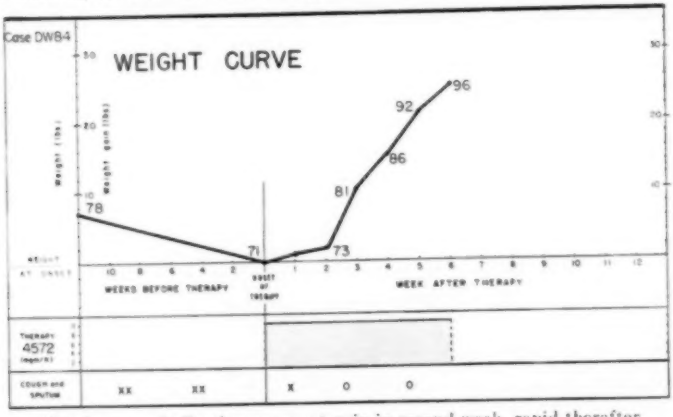
Weight curve showing beginning of ascent in second week in this instance.

FIG. 7. *Case 4* (F. W.)

Roentgenogram before onset of therapy showing extensive destruction of the left lung and slight exudative infiltrate into right upper lobe. Roentgenograms in January 1952 show little significant change despite unusual clinical improvement and recent tendency toward disappearance of acid-fast bacilli from sputum.

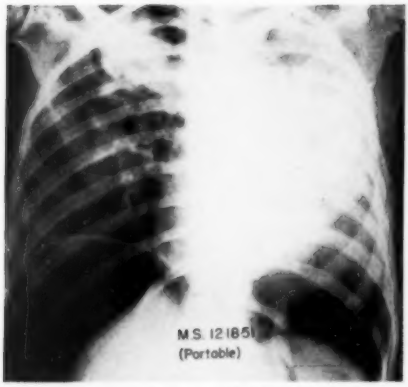


Temperature curve indicating persistently elevated temperature returning to normal on the third day after institution of therapy.

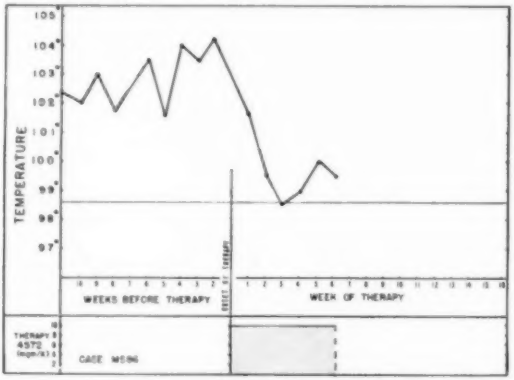


Weight curve indicating apparent gain in second week, rapid therafter.

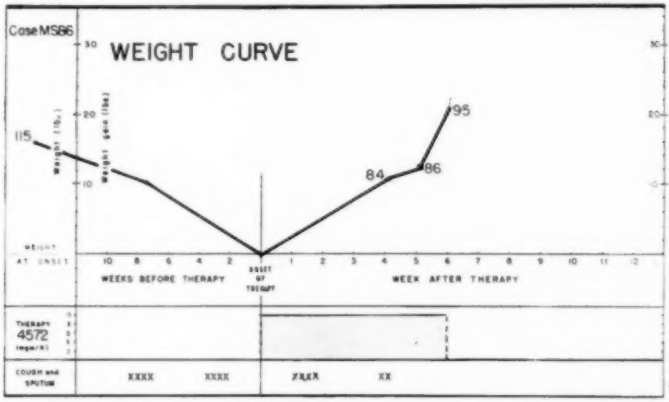
FIG. 8. *Case 5* (D. W.)



Portable bedside roentgenogram of December 18, 1951 showing intense destructive process throughout most of the left lung and caseous cavernous disease within the right upper lung field. Residuum of pneumoperitoneum, abandoned in November 1951, is seen.



Temperature curve, recorded in weeks, showing return to approximate normalcy in about the fourth week.



Graphic representation showing immediate response of weight to therapy.

FIG. 9. *Case 6* (M. S.)

Case 6 (M. S.). This 21-year-old Negro female was admitted to Sea View Hospital on June 28, 1951, soon after the onset of her pulmonary tuberculosis. She was treated with two courses of streptomycin, totaling 175 gm., and concurrent PAS for five months. Pneumoperitoneum was instituted on October 8, 1951. None of this therapy altered her rapidly progressive downhill course, and by December 1951 her condition was critical. She was highly toxic, with marked anorexia and weakness; she was apathetic and amenorrheic. She was continuously febrile, with temperatures ranging from 103° to 104°F. She had a severe cough productive of approximately 720 cc. of sputum daily which was highly positive for tubercle bacilli. She had lost 40 pounds in weight, and roentgenographic examination showed extensive bilateral caseous-pneumonic tuberculosis

Therapy with the isopropyl derivative (Marsilid) was begun on December 5, 1951 at a dosage level of 10 mg. per kg. per day. There was no particular improvement, and the patient continued febrile, toxic, and dehydrated. Physical examination revealed signs suggesting concomitant bronchopneumonia, and it was believed that this was nontuberculous, complicating her extensive pulmonary tuberculosis. Therefore, in addition to her antituberculous therapy, she was given intravenous fluids, terramycin, and penicillin. This therapy caused rapid improvement in her condition and was continued for three days. The temperature was not significantly altered but clinical improvement suggested elimination of secondary infection. Further improvement was rapid, and after four weeks of therapy with the isopropyl derivative (Marsilid), toxicity had been largely eliminated and temperatures averaged approximately 100°F.

At present the patient is afebrile, with minimal cough and expectoration although the sputum remains positive for tubercle bacilli. A roentgenogram on January 7, 1952 revealed no significant change in comparison with pre-treatment films. There has been a complete reversal, however, of her clinical state from that present before therapy. From an almost moribund condition, this patient is now afebrile, rapidly gaining weight, has an excellent appetite, and feels "marvelous" (figure 9).

DISCUSSION

The need for a large control series in the evaluation of therapy in tuberculous disease has often been debated. The use of such a control series was considered at the outset of the present study but was discarded as unnecessary so long as the group under observation was of sufficient magnitude. The literature on the fate of the febrile, acutely toxic, caseous-pneumonic tuberculous lesion is ample. Extensive experiences at Sea View Hospital confirm the impression that these cases do badly. This is especially true of the young Negro female. Toxic courses with hectic temperatures are often combined with intestinal and laryngeal tuberculosis, and the course is frequently one of unchecked progression, emaciation, and death. This has been modified sharply by antituberculous chemotherapy but, when these agents have failed, the course is ultimately the same and the outcome is merely delayed. Such cases constitute a large percentage of the total study on this service, and the current report relates experiences with 44 febrile members of the group adjudged to have caseous-pneumonic tuberculosis. As noted, in 41 of the 44, previous antituberculous chemotherapy had failed to control the pulmonary disease.

For rapid evaluation of the hydrazine derivatives of isonicotinic acid, this type of caseous-pneumonic lesion represents ideal material. To be sure, the

pulmonary disease revealed by roentgenography is in large measure massive and irreversible, so that initial evaluations cannot be made so readily by sputum examinations and by roentgenography as in less advanced disease. The toxicity of the disease, however, as shown in the present study, can be sharply and rapidly curtailed, and it is on this feature that preliminary emphasis has been placed. The extraordinary and rapid weight gains (average, 16.5 pounds for males, 22.0 pounds for females in 8.8 weeks), the resurgent appetites which exceed the previous known normals, the rapid return of temperature to normal (in some instances almost by crisis), and the restoration of energy suggest an adaptation of the patient's metabolic status to a new environment free of the depressive influence of systemic tuberculosis. Most encouraging in this respect has been the fact that this has occurred equally impressively in previous antibiotic failures and in *de novo* cases.

The rapidity of response implies, but does not prove, that a specific bactericidal effect may be operative, as suggested in animals by Grunberg and Schnitzer (4). Further bacteriologic study will be required in this connection. Temperature changes at times have been so dramatic as to suggest an antipyretic effect. But the few days' delay before specific temperature drop, the concomitancy of other clinical response, and the failure of the temperature to resume high levels on discontinuance of therapy indicate, rather, a specific antituberculous effect.

Early observations indicated that the nervous system was being influenced. Alertness, a tendency toward hyperreflexia in some instances, "nervousness," and occasional insomnia have suggested central stimulation. Constipation, dryness of the mouth, and bladder sphincter hypertonia have suggested an effect on the autonomic system. That this is probably not parasympathetic blockage has been suggested by an unusually severe reaction, implying overstimulation of the sympathetic system, in a patient who had been given 22.5 mg. of ephedrine sulfate. This will be reported in detail elsewhere. Similarly, symptoms have been ameliorated by the use of Prostigmine, on occasion.

It is the writers' impression that vertigo, frequently noted, is vasomotor in origin. Blood pressure drops of 20 to 30 mm. systolic and 10 to 15 diastolic, associated with elevated pulse rates by 30 beats per minute, have been observed during these episodes. These effects have been transitory and customarily have occurred at one-half to one and one-half hours after ingestion of the medication. The blood pressure has not been elevated in any instance, indicating that the hypothetical sympathetic stimulation has been unaccompanied by pressor activity. These are simple preliminary speculations which require thorough investigation in the pharmacology laboratories.

The question of proper dosage is still entirely unsettled. In the present study, the drug has been arbitrarily administered in three divided daily doses after meals. It was believed that a slightly higher incidence of vertigo occurred with administration before meals but this was not confirmed on statistical scrutiny. The concentrations of drug attained in the blood are currently being studied, but no statement can be made at this time. It is possible that such analysis will not be of material assistance in determining dosage schedules; it is equally possible

that the maintenance of high blood concentrations by three- or four-hour administration schedules will provide the greatest therapeutic benefit.

It must also be emphasized that the total optimal daily dosage per kilogram has not been determined. Therapeutic benefit has been noted on daily doses of 1 mg. of the glucoside derivative per kg., 1 mg. of the hydrazide per kg., and 1 mg. of the isopropyl derivative per kg. This benefit was progressively enhanced on daily doses of 2, 4, and 10 mg. of the isopropyl derivative per kg. and by 2 and 4 mg. of the isonicotinic acid hydrazide per kg. At present further trials are in progress which are designed to evaluate other dosages of these two compounds. Reference to the toxicity tables will show that increases in side effects may be anticipated with increasing dosages. Of interest has been the belated observation in the present study that in many instances the doses were spontaneously diminishing. A weight gain of 50 pounds in a 100-pound patient automatically reduces the dose by 33⅓ per cent.

A definitive statement concerning the relative values of the three drugs under investigation cannot be rendered at this time. On the basis of the present observations, however, it is believed that the glucoside derivative is least useful. Instability, need for refrigeration, and need for administration by injection diminished the versatility of this derivative. Further comments in this regard are reported elsewhere. A nonstatistical estimate of the therapeutic efficacy of this drug suggests that its responses are neither as profound nor as sudden as those of the other two.

At this stage of the present investigations, isonicotinic acid hydrazide (Rimifon) and the isopropyl derivative (Marsilid) appear to be nearly equal in effectiveness. Reference to the tables suggests that the isopropyl derivative (Marsilid) in the higher dosages exerts rapid, profound effect and, simultaneously, a higher incidence of toxic reactions during the first four weeks of therapy. Isonicotinic acid hydrazide (Rimifon) exerts less "acute" toxicity and an almost equal therapeutic response. It should be pointed out, however, that preliminary animal studies (6) suggest that long-term toxicity may occur at lower dosages, a feature which evokes greater caution. Designation of the definitive drug may require several years of study.

Tuberculosis of the tongue has been under therapy with isonicotinic acid hydrazide (Rimifon) and the isopropyl derivative (Marsilid) in 2 cases. The encouraging response will be reported in detail at another time.

Likewise, a statement will be forthcoming from the Ear, Nose and Throat Service regarding tuberculosis of the larynx. Suffice it to say that the present clinical observations, entirely apart from the specific laryngoscopic observations, indicate healing of the same magnitude as has been noted with streptomycin therapy.

The gastrointestinal lesions cannot now be tabulated. It is known from studies of the pathologic material at Sea View Hospital (13) that most patients with laryngeal lesions, and many without, have ulcerations of the intestinal tract, particularly in the ileocecal area. Tchertkoff and Green (14) showed that a febrile course of two or more months in a patient with sputum positive for tubercle

bacilli and with open cavities almost invariably indicated the presence of this type of lesion. Since most of the present cases have been febrile, a high percentage of gastrointestinal tuberculosis must have been present. No clinical or roentgenographic data are available to prove or disprove this assumption, but the clinical course supports the contention that a beneficial effect was obtained from this therapy.

No statement relating to the emergence of drug-resistant strains of tubercle bacilli can be made at this time. Serial cultures will be studied for the development of resistance. Entirely from the clinical point of view, however, no clear-cut evidence suggesting such resistance has been encountered in patients under therapy for more than three and one-half months. The patients continue to gain weight, retain their appetites, energy, and sense of well-being, and have had no resumption of cough and expectoration. These observations are believed to be significant, since many of these patients continue to discharge tubercle bacilli in the sputum in the presence of destroyed lungs. In the writers' experience, such patients under streptomycin therapy have usually exhibited resumption of symptoms with the emergence of drug-resistant strains of tubercle bacilli. Should the theories of adaptation be correct, ultimate resistance to the hydrazine derivatives of isonicotinic acid may be anticipated.

Drug reactions of the hypersensitivity type have not been seen. Five patients treated for seven weeks with the glucoside derivative were re-treated with the same drug six weeks after discontinuance. No allergic response was noted clinically, nor was eosinophilia found on examination of the blood smear. Two additional patients have had the isopropyl derivative (Marsilid) discontinued, with resumption of therapy three and four weeks later without allergic reaction. None of the patients under isonicotinic acid hydrazide (Rimifon) therapy have had discontinuance and resumption.

Dyspnea of minor degree has been noted in several instances. This was invariably seen in patients experiencing weight gain and concomitant increase in physical activity. The dyspnea was not deemed to be disproportionate to these factors.

Indulgence in speculation in this preliminary report does not seem improper. The ultimate therapy for the tuberculous lesion is obviously speculative. Should the factors of the emergence of drug-resistant tubercle bacilli remain of limited or of no significance, it is visualized that there might be a divergence from the current tendency to administer tuberculotherapy in courses and a return to therapy in accordance with the pathology of the individual case. The cases chosen for study in the present investigation obviously call for long-term therapy and many, even under the most sanguine circumstances, will require surgery for the correction of the residual pathology. No minimal cases have been studied, but for these an optimistic outlook seems justified.

On the basis of the findings presented here and elsewhere (15), it is believed that further investigation of the hydrazine derivatives of isonicotinic acid is warranted.

SUMMARY AND CONCLUSIONS

Forty-four cases of acute febrile, progressive caseous-pneumonic tuberculosis have been treated with two hydrazine derivatives of isonicotinic acid, (a) 1-isonicotinyl -2- isopropyl hydrazine (Marsilid) and (b) isonicotinic acid hydrazide (Rimifon). Therapy to date has ranged from four to fifteen weeks.

All patients experienced rapid and marked reversal of their original toxic states, as evidenced by gain in weight, return of appetite, defervescence, and a sharp return in sense of well-being.

Cough and expectoration have been eliminated or markedly reduced.

Sputum bacillary contents have been reduced in 38 cases and, in 8 cases, examinations for acid-fast bacilli on stained smears have been repeatedly negative.

On roentgenographic examination, reduction in cavity size has occurred in 17 cases and apparent diminution in exudate has occurred in 5 cases.

Therapeutic effects of isonicotinic acid hydrazide (Rimifon) after four weeks at 4 mg. per kg. daily are roughly equivalent to 1 - isonicotinyl - 2- isopropyl hydrazine (Marsilid) at the same dosage and for the same period.

The incidence of early side reactions is moderately higher with the isopropyl derivative (Marsilid) therapy at comparable dosages although, from tentative and preliminary animal studies, isonicotinic acid hydrazide (Rimifon) might have a higher potential delayed toxicity.

The hydrazine derivatives of isonicotinic acid exert an impressive therapeutic effect upon the course of acute caseous-pneumonic tuberculosis in humans.

SUMARIO Y CONCLUSIONES

Los Derivados Hidracínicos del Acido Isonicotínico (Rimifón, Marsilid) en el Tratamiento de la Tuberculosis Cáseo-Neumónica Evolutiva Activa

Cuarenta y cuatro casos de tuberculosis cáseo-neumónica evolutiva febril aguda han sido tratados con dos derivados hidracínicos del ácido isonicotínico: (a) 1-isonicotinil-2-isopropil hidracina (Marsilid) y (b) hidracida del ácido isonicotínico (Rimifón). La terapéutica hasta la fecha ha durado de cuatro a quince semanas.

Todos los enfermos manifestaron rápido y notable viraje de sus primitivos estados tóxicos, según evidenciaron el aumento de peso, vuelta del apetito, defervescencia y decidido retorno de una sensación de euforia.

La tos y la expectoración han sido eliminadas o reducidas en forma pronunciada.

El contenido bacilar del esputo ha sido rebajado en 38 casos, y en 8, los exámenes en busca de bacilos ácidorresistentes en frotes teñidos han sido negativos repetidamente.

Conforme al examen radiográfico, ha habido disminución del tamaño de las cavernas en 17 casos y aparente disminución del exudado en 5.

Al cabo de cuatro semanas a 4 mg. diarios por kg. de peso, los efectos terapéuticos de la hidracida del ácido isonicotínico (Rimifón) vienen a ser equivalentes a los de la 1-isonicotinil-2-isopropil hidracina (Marsilid) a la misma dosis y durante el mismo período de tiempo.

La incidencia de reacciones laterales tempranas es moderadamente mayor con la terapéutica con el derivado isopropílico (Marsilid) a dosis comparables, aunque, juzgando por los estudios en animales, la hidracida del ácido isonicotínico (Rimifón) acaso posea una toxicidad tardía potencial mayor.

Los derivados hidracínicos del ácido isonicotínico ejercen un imponente efecto terapéutico sobre la evolución de la tuberculosis cáseo-neumónica aguda en el hombre.

RESUME ET CONCLUSIONS

Le dérivé hydrazine de l'acide isonicotinique (Rimifon, Marsilid) dans la pneumonie caséeuse tuberculeuse évolutive

Quarante-quatre cas aigus de pneumonie caséeuse tuberculeuse évolutive avec fièvre ont été traités avec deux dérivés hydrazinés de l'acide isonicotinique, (a) le 1-isonicotinyl-2-isopropyl hydrazine (Marsilid) et (b) l'hydrazide de l'acide isonicotinique (Rimifon). A ce jour, la durée des traitements a été de quatre à quinze semaines.

Chez tous les malades il s'est produit une transformation rapide et marquée des conditions toxiques, qui existaient à l'origine, comme l'ont montré l'augmentation du poids, le retour de l'appétit, la chute de la température et une brusque reprise de l'état général.

La toux et l'expectoration ont été éliminées ou réduites de façon marquée.

Le contenu en bacilles des expectorations a diminué dans 38 cas et, dans 8 cas, la recherche des bacilles acido-résistants sur les étalements colorés s'est montrée constamment négative.

L'examen des clichés radiographiques indique une réduction du volume des cavernes dans 17 cas et, dans 5 cas, la diminution de l'exudat s'est manifesté. Au bout de quatre semaines les effets thérapeutiques de l'hydrazide de l'acide isonicotinique (Rimifon) à la dose de 4 mg. par kg. par jour sont à peu près équivalents à ceux de 1-isonicotinyl-2-isopropyl hydrazine (Marsilid) aux mêmes doses et pendant la même période.

A doses comparables, l'incidence de réactions secondaires immédiates est légèrement plus élevée avec la thérapie par le dérivé isopropyl (Marsilid), comme le montre l'expérimentation sur l'animal; il semble que l'hydrazide de l'acide isonicotinique possède un potentiel de toxicité retardée plus élevé.

Les dérivés hydrazinés de l'acide isonicotinique exercent une action thérapeutique impressionnante sur le cours de la pneumonie caséeuse tuberculeuse aiguë chez l'homme.

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THE ABSORPTION, DISTRIBUTION, EXCRETION, AND SHORT-TERM TOXICITY OF ISONICOTINIC ACID HYDRAZIDE (NYDRAZID[1]) IN MAN[2, 3]

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INTRODUCTION

Isonicotinic acid hydrazide (Nydrazid) has been demonstrated by Bernstein, Lott, Steinberg, and Yale of the Squibb Institute to have a considerable and unique inhibitory effect on the growth of *Mycobacteria in vitro* and to exert an impressive degree of antituberculous activity in experimentally infected mice (1). Systematic studies of the pharmacology of this compound were conducted by Rubin, Hassert, Thomas, and Burke (2) who defined the acute and chronic toxicities of the drug for several animal species, including dogs. They found that isonicotinic acid hydrazide was well tolerated by dogs for observation periods of three to four months when administered orally in doses which provided theoretically effective concentrations in the plasma. As a consequence of these observations, an investigation in man of the pharmacodynamics and antituberculous activity of isonicotinic acid hydrazide was started in November 1951 on the New York Hospital-Cornell Medical Service. Observations made in this study, on the pharmacology and toxicity of the drug when administered to patients with pulmonary tuberculosis, form the basis of the present report.

PLAN OF INVESTIGATION

Patients: The patients chosen for study were all adults with pulmonary tuberculosis classified as far advanced or moderately advanced by the criteria listed in DIAGNOSTIC STANDARDS of the National Tuberculosis Association (1950 edition). With a few exceptions, the patients had had long courses of streptomycin and para-aminosalicylic acid (PAS) and were discharging tubercle bacilli which were insusceptible to streptomycin *in vitro*. The type and timing of the clinical, bacteriologic, and roentgenographic observations made of the course of the tuberculous infection during isonicotinic acid hydrazide administration were identical with those listed in previous publications (3, 4).

Drug: The isonicotinic acid hydrazide[4] used was a highly purified crystalline powder incorporated into capsules containing 10.0 mg. or 25.0 mg.

Dosage regimen: For continued administration, the isonicotinic acid hydrazide was given

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[1] Proprietary name, E. R. Squibb & Sons.

[2] From the Department of Medicine, New York Hospital-Cornell Medical Center, New York, New York.

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[4] Generously supplied as Nydrazid by E. R. Squibb and Sons, New York, N. Y.

orally in a total daily dose of 3 mg. per kg. divided into two doses at approximately twelve-hour intervals. Deviations from this regimen were made in certain individual experiments and will be noted below.

Collection of specimens: Following a single oral dose of isonicotinic acid hydrazide, specimens of venous blood were drawn at appropriate intervals under aseptic precautions and placed in sterile test tubes containing enough potassium oxalate to produce a final concentration of 0.3 per cent. The blood was centrifuged at 3,000 r.p.m. for twenty minutes, and the supernatant plasma was removed for analysis. The total twenty-four-hour output of urine was collected in fractions which were measured and analyzed. Specimens of pleural fluid, cerebrospinal fluid, sputum, saliva, and feces were obtained by the customary techniques.

Chemical assay of isonicotinic acid hydrazide in body fluids: Determinations of the concentration of isonicotinic acid hydrazide in the various body fluids were made by the technique of Kelly and Poet (5). Samples were extracted into an isoamyl alcohol-ether-ammonium sulfate system which was redistributed with a 0.1 *N* HCl phase. This phase was reacted with *p*-dimethylaminobenzaldehyde, and the color produced was measured in a Beckman spectrophotometer at 450 mu, using 1 cm. Corex cells against a sample of 0.1 *N* HCl. The values of the samples containing unknown amounts of isonicotinic acid hydrazide were adjusted by the appropriate known zero blank and compared with standard recovery values for blood, urine, cerebrospinal fluid, pleural fluid, ascitic fluid, saliva, and sputum. The sensitivity of the method was obtained by the following procedure.

The minimum, median, and maximum absorptions per γ per ml. of biologic material were obtained by performing multiple determinations from prepared standards; and the minimum, median, and maximum absorptions per ml. of control biologic material were also obtained. Calculations were made with the following equations:

$$\text{Minimal detectable concentration} = \frac{\text{maximum absorption of biologic material per ml.}}{\text{minimum absorption per } \gamma \text{ per ml.}}$$

$$\text{Sensitivity in } \gamma \text{ per ml.} = \frac{\text{maximum deviation from the median absorption of the biologic control and maximum deviation per } \gamma \text{ of drug per ml. of biologic material}}{\text{median absorption per } \gamma \text{ of drug per ml. of standard biologic material}}$$

It was possible to use these equations because of the linear relation of per cent absorption to concentration for the range of concentrations encountered.

Because urine concentrations covered a great range, two dilution factors were employed. The values for both methods are listed in table 1.

Renal clearance of isonicotinic acid hydrazide: The following equation was used in calculating clearance:

$$\text{Clearance} = \frac{\text{urinary excretion in } \gamma \text{ per minute}}{\text{plasma concentration in } \gamma \text{ per ml.}}$$

The values selected for these calculations were chosen for a time period after the plasma concentration had begun to decline. Large variations in absorption, as evidenced by a slow or irregular rise or fall in plasma concentrations, necessitated excluding 2 patients from these calculations.

It is interesting to observe that the one patient in whom the plasma concentration was high enough to be measured directly at twenty-four hours exhibited a comparable concentration when calculated from his urinary excretion and clearance during the twenty-fourth hour.

Antituberculous activity of plasma: The antituberculous activity of plasma obtained from patients at various intervals after ingestion of isonicotinic acid hydrazide was tested *in vitro* against *M. tuberculosis* (H37Rv) in liquid medium containing 0.05 per cent albumin and 0.0002 per cent Tween 80. An inoculum of the culture containing approximately 50,000

viable units was used and the end point of the tests was determined by gross inspection after fourteen days' incubation at 37°C.

Tests of renal and hepatic function: The urea clearance was calculated according to the formula of Moeller, McIntosh, and Van Slyke (6), and the plasma proteins were measured by the method described by Kingsley (7). The sulfobromphthalein sodium retention (8) and the thymol turbidity tests (9) were used in the evaluation of hepatic function.

Protocol of the investigation: The subjects remained in bed throughout the period of the investigation and were observed daily for any evidences of drug toxicity. Particular attention was paid to the detection of any evidences of excitation of the nervous system. Immediately prior to the institution of isonicotinic acid hydrazide, the status of renal, hepatic, and hematologic functions was determined. Total leukocyte and differential counts were performed twice weekly, together with examinations of the urine. The hematocrit, erythrocyte sedimentation rate, erythrocyte counts, and hemoglobin concentrations were determined weekly. Chemical determinations of the blood performed at two-week intervals included measurements of CO_2 combining power, chloride concentrations, blood sugar (fasting), calcium and phosphorus concentrations, total protein, and albumin-globulin ratios. Renal function was estimated every two weeks after the start of isonicotinic acid

TABLE 1

THE ACCURACY OF THE CHEMICAL ASSAY METHOD WHEN APPLIED TO VARIOUS BIOLOGIC MATERIALS

BIOLOGIC MATERIAL	MINIMAL DETECTABLE CONCENTRATIONS	SENSITIVITY
	γ *per ml*	γ *per ml*
Plasma	0.4	±0.25
Urine 1/4A	10.0	±8.0
1/20B	13.0	±7.0
Feces	10.0	±10.0
Cerebrospinal fluid	0.25	±0.25
Pleural fluid	0.4	±0.25
Saliva	0.25	±0.25

hydrazide by urea clearance and blood urea nitrogen determinations. Hepatic function was estimated every two weeks by bromsulfonphthalein retention, bilirubin concentration, thymol turbidity, and alkaline phosphatase activity. Cardiac function was studied by pulse and blood pressure charts and electrocardiography.

OBSERVATIONS

Absorption, Distribution, and Excretion

Plasma concentrations: A single oral dose of 3 mg. of isonicotinic acid hydrazide (Nydrazid) per kg. was administered to each of 6 adult patients with normal renal function. The range of the individual doses was 140 to 200 mg. of drug. The concentrations of isonicotinic acid hydrazide attained in the plasma at various intervals after ingestion are represented in figure 1. As may be seen in the figure, the drug was absorbed promptly and the maximum concentrations (1.3 to 3.4 γ per ml.) were attained in the plasma between one and six hours after ingestion. Drug concentrations at or above the minimal concentration detectable by the method used (0.40 γ per ml.) persisted in the plasma for six to twenty-four hours after administration.

In another set of observations, the plasma concentrations attained following a morning dose of 1.5 mg. per kg. were determined in 5 patients who had been receiving this dose twice daily for a minimum period of one week. These data are presented in table 5. As may be seen, the maximum concentrations among the

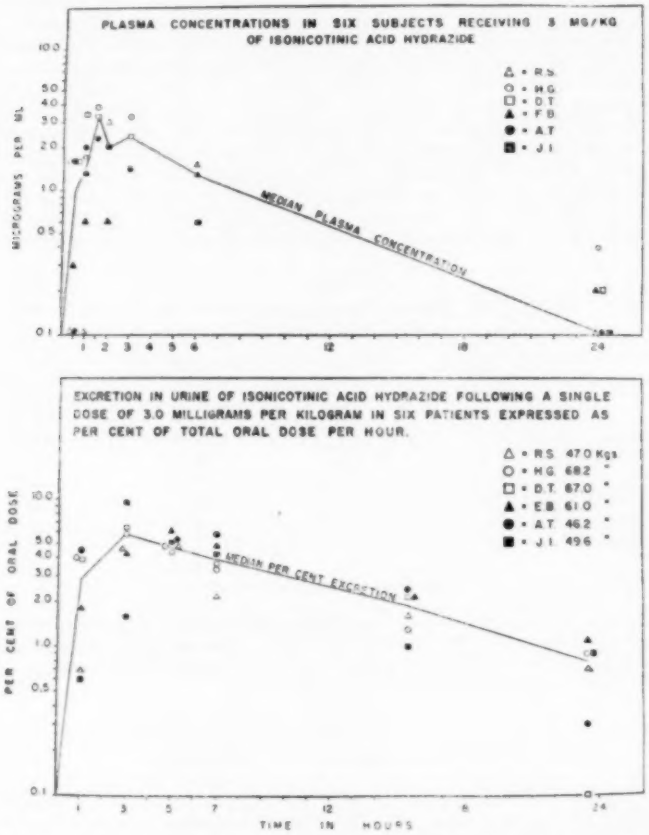
PLASMA CONCENTRATIONS IN SIX SUBJECTS RECEIVING 3 MG/KG OF ISONICOTINIC ACID HYDRAZIDE
MICROGRAMS PER ML
MEDIAN PLASMA CONCENTRATION
EXCRETION IN URINE OF ISONICOTINIC ACID HYDRAZIDE FOLLOWING A SINGLE DOSE OF 3.0 MILLIGRAMS PER KILOGRAM IN SIX PATIENTS EXPRESSED AS PER CENT OF TOTAL ORAL DOSE PER HOUR.
PER CENT OF ORAL DOSE
MEDIAN PER CENT EXCRETION
TIME IN HOURS

FIG. 1. The absorption and simultaneous urinary excretion of isonicotinic acid hydrazide following administration of a single dose of 3.0 mg. per kg. to each of 6 patients.

individual patients ranged between 0.60 γ and 1.48 γ per ml., and the drug could be detected in the plasma for three to seven hours after administration.

In 7 patients who were receiving isonicotinic acid hydrazide daily in a total dosage of 3.0 mg. per kg. (administered in two doses), weekly determinations of the plasma concentrations of drug were made approximately three hours

after the morning dose. From these data, which are presented in table 6, no evidence was obtained of accumulation of the drug on long-continued administration.

Renal clearance and excretion in urine: In figure 1 may be seen the per cent of the total 3 mg. per kg. dose of isonicotinic acid hydrazide which was excreted per hour by the 6 patients whose plasma concentrations are indicated in the graph. The determinations of the concentrations of drug in plasma and in urine were made during the same twenty-four-hour experimental period. During this period, the individual patients excreted in the urine between 47.8 and 70.7 per cent of

TABLE 2

THE ABSORPTION OF ISONICOTINIC ACID HYDRAZIDE

PATIENT	TOTAL ORAL DOSE	PLASMA CONCENTRATIONS OF ISONICOTINIC ACID HYDRAZIDE IN γ PER ML. IN 6 PATIENTS FOLLOWING ADMINISTRATION OF A SINGLE ORAL DOSE OF 3.0 MG. PER KG.						
		½ hour	1 hour	1¼ hours	2 hours	3 hours	6 hours	24 hours
R. S.	140 mg.	0.1	0.0	—	3.0	—	1.5	0.0-0.4
H. G.	200 mg.	1.6	1.7	3.9	—	3.3	—	0.4
D. T.	200 mg.	1.6	3.4	3.3	—	2.4	—	0.0-0.4
F. B.	180 mg.	0.3	0.6	—	0.6	—	1.3	0.0-0.4
J. I.	150 mg.	0.0	1.3	—	2.0	—	0.6	0.0-0.4
A. T.	140 mg.	1.6	2.0	2.3	—	1.4	—	0.0-0.4

TABLE 3

THE URINARY EXCRETION OF ISONICOTINIC ACID HYDRAZIDE

PATIENT	TOTAL ORAL DOSE	TOTAL PER CENT ORAL DOSE RECOVERED IN URINE	URINARY EXCRETION OF ISONICOTINIC ACID HYDRAZIDE IN PER CENT OF TOTAL ORAL DOSE PER HOUR IN 6 PATIENTS FOLLOWING ADMINISTRATION OF A SINGLE ORAL DOSE OF 3.0 MG. PER KG.					
			0-2	2-4	4-6	6-8	8-23	23-24
R. S.	140 mg.	47.8	0.7	4.5	4.6	2.2	1.6	0.7
H. G.	200 mg.	55.6	4.0	5.8	4.7	3.4	1.3	0.9
D. T.	200 mg.	69.6	3.9	6.4	4.5	3.6	2.2	0.03
F. B.	180 mg.	68.1	1.8	4.4	6.1	4.8	2.2	1.1
J. I.	150 mg.	54.3	0.6	9.4	4.9	4.2	1.0	0.9
A. T.	140 mg.	70.7	4.5	1.8	5.3	5.8	2.4	0.3

the dose administered. In general, as may be seen in figure 1, after the first two hours, the rate of excretion of the urine was reasonably constant, and the curve of the excretion of the drug in the urine was similar to the curve of the plasma concentrations in the same patient. It is of interest that, during the twenty-fourth hour after the administration of the drug, between 0.03 and 1.1 per cent of the total oral dose was excreted in the urine. Although with one exception (patient H. G.) with the technique used it was not possible to demonstrate the presence of the drug in the plasma at this time (twenty-fourth hour), the amounts excreted in the urine presumably reflect the presence of substantial, albeit chemically undetectable, quantities in the plasma.

The data permitted calculation of the renal clearance of isonicotinic acid hydrazide in 4 of the 6 patients. The clearances ranged from 60 to 156 as listed in table 4. From these values it was possible to make a calculation of the concentrations of isonicotinic acid hydrazide present in the plasma during the twenty-fourth hour. The results of these calculations are presented in table 4 where the values predicted from calculation of plasma clearance of the drug are compared with the values actually obtained by chemical assay of the blood at that time.

Excretion by other routes: A substantial concentration of isonicotinic acid hydrazide (1.43 γ per ml.) was excreted in the saliva of the one patient tested three hours after ingestion of a 1.5 mg. per kg. dose. Total stool collections were

TABLE 4

PLASMA CONCENTRATIONS OF ISONICOTINIC ACID HYDRAZIDE CALCULATED FROM URINARY EXCRETION

PATIENT	CLEARANCE	PLASMA CONCENTRATION AT 23½ HOURS CALCULATED FROM CLEARANCES	PLASMA CONCENTRATION MEASURED CHEMICALLY
		γ per ml	*γ per ml*
R. S.	63	0.24	0.0-0.4
H. G.	60	0.48	0.4 ± 0.25
D. T.	88	0.05	0.0-0.4
J. I.	156	0.11	0.0-0.4

TABLE 5

PLASMA CONCENTRATIONS OF ISONICOTINIC ACID HYDRAZIDE IN MICROGRAMS PER MILLILITER IN 5 PATIENTS ON CONTINUOUS DOSAGE OF 3.0 MG. PER KG. DIVIDED INTO TWO DOSES PER DAY

PATIENT	3 HOURS	7 HOURS
R. S.	0.79	0.45
H. G.	1.48	0.78
F. B.	0.90	0.0-0.4
J. I.	0.76	0.0-0.4
A. T.	0.60	0.0-0.4

attempted in only 2 patients (F. B., H. G.), the first stool passed after ingestion of the drug was assayed. Concentrations of 40.4 and 79.4 γ of isonicotinic acid per gram of stool were found in specimens passed twenty-five and thirty-six hours after administration of the drug. Attempts to measure the concentration of the drug in the sputum were unsuccessful because of great variation in the control determinations of sputum from untreated patients which raised the minimum detectable concentration to more than 5 γ per ml.

Distribution into body compartments: The transfer of isonicotinic acid hydrazide into the cerebrospinal fluid following oral administration of the drug was studied in 9 patients (4 adults, 5 children). Three of the patients presented no evidence of meningeal infection. A fourth patient had been previously treated

for tuberculous meningitis but had displayed no clinical or cerebrospinal fluid evidence of active meningeal infection for a period of three months before receiving the isonicotinic acid hydrazide. The remaining 5 patients all had active tuberculous meningitis at the time of the investigation.

The dosages of isonicotinic acid hydrazide, the time relationships of the withdrawal of the cerebrospinal fluid to the ingestion of the drug, and the concentrations of drug in cerebrospinal fluid and plasma are presented in table 9.

As may be seen (table 9), appreciable concentrations of the drug were present in the cerebrospinal fluid of all patients. The earliest withdrawal of fluid after the first administration of the drug was made at three and one-half hours in one of the 4 patients without active meningitis, and at three and one-fourth hours in one of the 5 patients with active meningitis. In all 9 of these patients, appreciable concentrations of isonicotinic acid hydrazide were present in the fluid.

TABLE 6

PLASMA CONCENTRATION OF ISONICOTINIC ACID HYDRAZIDE IN PATIENTS RECEIVING 3.0 MG. PER KG. PER DAY DIVIDED IN TWO EQUALLY SPACED ORAL DOSES PER DAY
The Concentrations Were Obtained Three Hours After the First Oral Dose on the Day Indicated

PATIENT	TIME IN DAYS				
	7	14	21	28	35
R. S.	0.79	0.99	1.21	1.00	1.12
H. G.	1.48	1.23	1.82	1.55	1.48
F. B.	0.90	0.68	0.83	—	—
J. I.	0.76	0.86	0.99	0.90	0.97
A. T.	0.60	0.42	0.70	—	—
D. T.	1.20	1.59	—	—	—
E. S.	1.33	0.64	1.18	—	—

A comparison of the drug concentrations in the meningitis and non-meningitis cases is not possible because of the difference in the dosages in the two groups.

Without regard to the time of withdrawal of the cerebrospinal fluid, the concentrations of drug in the fluid of the 5 patients with active meningitis (1.77 to 4.10 γ per ml.) were substantially higher than the concentration (0.06 γ per ml.) which completely inhibits the H37Rv strain of *M. tuberculosis* for fourteen days *in vitro*.

Repeated examination of the cerebrospinal fluid in the 5 patients with active meningitis at weekly intervals showed no significant change when the cerebrospinal fluid was obtained at approximately the same time after the last oral dose as in the preceding weeks (table 9).

Distribution of isonicotinic acid hydrazide into the pleural cavity was studied in one patient with tuberculous empyema who had never previously received the drug. At two hours after ingestion of a single dose of 2.0 mg. per kg. (total dose, 160 mg.), a drug concentration of 0.0 to 0.4 γ per ml. was found in the pleural exudate.

TABLE 7

SUMMARY OF TOXICITY DATA FROM PATIENTS RECEIVING ISONICOTINIC ACID HYDRAZIDE

	UNIT OF MEASURE	R. S.		H. G.		D. T.		F. B.		A. T.		J. I.		E. S.	
		0 Week	12 Week	0 Week	6 Week	0 Week	6 Week	0 Week	6 Week	0 Week	6 Week	0 Week	6 Week	0 Week	4 Week
Hemoglobin	Grams per 100 cubic centimeter	11.0	13.5	14.0	13.5	15.5	13.5	14.5	13	12.0	12.0	15.0	15.0	13.5	15.5
Erythrocyte count	Million per cubic millimeter	3.6	5.2	3.9	4.4	4.9	4.6	4.7	4.4	3.8	3.5	4.6	4.6	4.0	4.3
Hematocrit	Per cent	36	43	38	42	42.9	43	43	40	36	36	40.5	44	43	44
Erythrocyte sedimentation rate	Millimeter per centimeter	55	43	30	44	46	44	47	49	55.5	54	26	6	14	8
Icterus index	Units	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5	0-5
Hemolysis	Present or absent	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leukocyte count	Thousands per cubic millimeter	10.4	8.7	10.0	8.4	6.5	6.2	12.2	11.6	18.0	20.6	12.5	7.1	7.0	12.1
Differential leukocyte count: Polymorphonuclear leukocytes															
(Band forms)	Per cent	27	3	4	2	5	7	6	7	36	16	11	0	4	1
(Mature forms)	Per cent	53	58	70	70	49	44	52	75	49	66	62	60	42	85
Lymphocytes	Per cent	17	25	16	20	40	41	8	9	6	9	18	25	39	11
Monocytes	Per cent	3	8	6	4	4	6	7	5	5	9	6	14	10	3
Eosinophils	Per cent	0	6	4	2	2	2	27	4	2	0	3	1	4	0
Basophils	Per cent	0	0	0	1	0	0	0	0	2	0	0	0	1	0
Weight	Kilograms	42.3	54.2	68.2	72.0	69.0	70.2	62.4	66.6	46.2	46.6	49.6	53.2	59.1	59.0
Alkaline phosphatase activity	Bodansky units	9.0	3.3	2.5	4.0	5.6	5.0	2.9	3.3	2.7	3.9	1.7	2.3	2.8	3.3

Sulfone bromphthalein retention	Milligram per cent	trace	0	9.4	9.4	8.1	3.3	3.9	0	2.4	3.8	1.3	3.1	0.8	0
Bilirubin	Milligram per cent	0.5	0.1	0.5	0.3	0.4	0.3	0.3	0.2	0.5	0.3	0.3	0.3	0.2	0.4
Thymol turbidity	Units	5	5	1	2	4	8	3	3	3	1	4	6	3	3
Blood urea nitrogen	Milligram per cent	14	22	17	14	11	12	10	8	5	8	14	14	14	16
Urea clearance															
First hour	Per cent	—	—	67.8	43	—	—	96.0	104	120	123	64.4	41	97.4	89
Second hour	Per cent	—	—	61.5	65.6	—	—	103	113	120	152	108	97	90.5	93

Toxicity

A total of 10 patients have received isonicotinic acid hydrazide in a daily dosage of 2 to 3 mg. per kg. for a period of one week. In 6 patients, the drug has been administered daily in a dose of 3 mg. per kg. in two divided doses for periods which range between six and sixteen weeks. No evidences of potentially serious toxicity have been encountered in any of the patients who have received the drug.[5]

Hematologic: The data on the various hematologic studies are presented in table 7. As may be seen in the table, there were no evidences that administration

TABLE 8

THE COMPARISON OF CHEMICAL AND BIOLOGIC ASSAY (WITH H37Rv) METHODS ON THE DETERMINATION OF ISONICOTINIC ACID HYDRAZIDE CONCENTRATIONS

CASE	CHEMICALLY	BIOLOGIC ASSAY
	Cerebrospinal Fluid	
	γ per ml	γ per ml.
F. E.	0.55	>1.3
A. T.	0.50	0.13-0.7
J. I.	0.55	0.13-0.7
R. M.	3.38	>5.0
E. J.	1.77	2.5 -5.0
R. J.	2.03	>5.0
E. B.	4.10	0.7 -1.3
B. Z.	1.95	1.3 -2.6
	Plasma	
A. T.	0.70	0.7 -1.3
J. I.	0.99	0.4 -0.7
H. G.	1.80	0.7 -1.3
F. B.	0.80	0.4 -0.7
E. S.	1.30	0.4 -0.7
R. S.	1.20	1.3 -2.6

of isonicotinic acid hydrazide in the dosage used was associated with any toxicity for the hematopoietic system. In one patient an eosinophilia of 15 per cent was noted, but this disappeared within three days despite the fact that drug administration was not interrupted.

Tests of renal and hepatic function: Serial tests of renal and hepatic function were performed on 7 patients who received isonicotinic acid hydrazide on the 3.0 mg. per kg. daily regimen for a minimum period of four weeks. The relevant

[5] An additional 8 patients, all children or young adults, have received isonicotinic acid hydrazide in a minimal daily dosage of 5 mg. per kg. for periods of four weeks (6 patients) and two weeks (2 patients) without evidences of toxicity. All but one of these patients are part of the meningeal-miliary study, mentioned elsewhere in the present paper, which is being conducted at Tuba City, Arizona, in collaboration with Dr. Charles M. Clark (U.S. P.H.S.) of the Indian Medical Service.

data are presented in table 7 from which it may be seen that in no instance were any disturbances of renal or hepatic function detected.[6]

Miscellaneous studies: Despite careful questioning and repeated neurologic examinations, no evidence of excitation of the central nervous system was detected in any of the patients during the period of observation on the daily dose of 3.0 mg. per kg. of isonicotinic acid hydrazide. Vertigo and dizziness were uniformly denied by all of the patients.

Antituberculous Acitivity of the Cerebrospinal Fluid and Plasma of Patients Receiving Isonicotinic Acid Hydrazide

Aliquots of cerebrospinal fluid and plasma were assayed both biologically (against H37Rv) and chemically. These values are compared in table 8. A comparison of the values shows that a reasonable correlation existed between the

TABLE 9

CONCENTRATIONS OF ISONICOTINIC ACID HYDRAZIDE IN THE PLASMA AND CEREBROSPINAL FLUID AFTER ORAL ADMINISTRATION

PATIENT	LAST ORAL DOSE	HOURS AFTER LAST ORAL DOSE	PLASMA CONCENTRATION	HOURS AFTER LAST ORAL DOSE	CEREBROSPINAL FLUID CONCENTRATION
	mg. per kg.		*mg. per kg.*		*mg. per kg.*
A. T.	1.5	5	0.40	5½	0.50
J. I.	1.5	5½	0.40	5¾	0.55
F. E.*	2.0	—	—	3½	0.55
J. S.	3.5	5	2.48	3¾	4.10
R. M.†	3.1	6¼	1.08	3½	3.38
E. J.†	2.9	6¼	0.61	3½	1.77
R. J.†	2.5	4½	2.00	3¾	2.03
E. B.†	2.8	4¼	2.40	4	4.10
B. Z.†	1.8	4½	1.42	4¼	1.95

* Previously treated tuberculous meningitis; no evidence of activity for several months before present study.

† Active tuberculous meningitis present at time of study.

two methods of assay. Moreover, the findings indicated that isonicotinic acid hydrazide was absorbed and distributed in both plasma and cerebrospinal fluid in a biologically active state. Protein binding was apparently a minor consideration since activity against *M. tuberculosis* H37Rv was present in media containing either 18 per cent plasma or 0.5 per cent bovine albumin.

DISCUSSION

On the basis of the above observations, it appears that isonicotinic acid hydrazide (Nydrazid) can be administered daily for periods of four to sixteen weeks to patients ill with pulmonary tuberculosis without evidence of serious toxic reactions. The daily dosage regimen (3.0 mg. per kg.) generally used in the present study was associated with plasma concentrations of the drug which are con-

[6] Considerable variation of urea clearance has been noted from this laboratory with these and untreated patients and is ascribed to technical difficulties.

siderably above those calculated to have been present in the therapeutic studies in mice reported by Bernstein and his associates (1). Moreover, the plasma concentrations of isonicotinic acid hydrazide attained in the present series of patients (less than 4.0 γ per ml.) were well below the drug concentration present in the dogs studied by Rubin and his associates (2) at the time evidences of toxicity developed. Finally, the biologic studies with plasma, cerebrospinal fluid, and tubercle bacilli in the present investigation indicate that the isonicotinic acid hydrazide distributed in the plasma and cerebrospinal fluid of man is present in an active form in terms of antituberculous activity *in vitro*.

It appears, therefore, that from the standpoint of distribution, maintenance of antimicrobial activity, and short-term tolerance, isonicotinic acid hydrazide in man displays properties which are highly desirable in an antituberculous drug.

The virtually complete absence of toxicity during a minimum period of four weeks (six weeks for 6 patients) of administration of isonicotinic acid hydrazide in approximately 3 mg. per kg. daily doses was an unexpected feature of the present study. It should be emphasized that this exceptionally good tolerance may well be deceptive. It is probable that instances of toxicity similar to those seen in dogs will be observed when the drug is administered at more frequent intervals, in a larger daily dosage, or for a longer period of time. In a few instances, much larger daily doses (10 to 15 mg. per kg.) were given for periods of only a few days without producing evidence of toxicity. Doses of this size, however, are known to be within the toxic range on continued administration to dogs though they are considerably lower than the acute toxicity dose for this animal. As a consequence, the use of such large doses is not advisable in humans save in very special circumstances and with very close observation.

It is well recognized that determinations of the concentration of a drug in the body fluids are seldom of value in the routine antimicrobial therapy of clinical infections. In the initial investigations of a new compound, however, such studies serve the purpose of providing a basis for the choice of dosage regimens. Moreover, when, as in the present case, the drug in question represents one of a potentially large series of similar compounds, studies of the concentration of drug attained in the body permit ready comparison of various derivatives. The degree of correlation between the chemical and biologic methods for assaying isonicotinic acid hydrazide in body fluids was sufficiently close so that the latter method should prove satisfactory for most purposes. The H37Rv strain of *M. tuberculosis* was used in the bioassays in the present study. The susceptibility of BCG to isonicotinic acid hydrazide *in vitro* is identical to that of H37Rv, however, and a method of bioassay with the use of BCG has been developed and will be published subsequently (10).

The rapid attainment of high concentrations of isonicotinic acid hydrazide in the cerebrospinal fluid in the presence of tuberculous meningitis suggests that high concentrations are also present in the extracellular fluid of the brain following oral administration of the drug. In the presence of meningitis, the cerebrospinal fluid concentrations of isonicotinic acid hydrazide represented 14 to 65 times the concentration which completely inhibits H37Rv *in vitro* for a fourteen-day period. Such pharmacologic behavior of a drug in respect to the central

nervous system is not necessarily an indication of high therapeutic activity. Nevertheless, the finding was considered to be sufficiently promising to warrant extending the therapeutic studies to meningeal and miliary tuberculosis in certain highly specialized circumstances.[7]

Substantial gains in appetite and body weight, reduction in fever, and favorable roentgenographic changes have been observed in certain of the patients. The interpretation of these events has been clouded, however, by the facts that most of the group had been subjected to the appetite-reducing effects of PAS immediately before the period of study, and defervescence has not occurred with uniformity. Moreover, the period of observation has also been too short to permit clinical or bacteriologic observations of any consequence on the highly important question of whether drug-resistant tubercle bacilli emerge in significant numbers in patients who receive isonicotinic acid hydrazide on continued administration.

Thus, despite the demonstrably high antituberculous activity of isonicotinic acid hydrazide in animals and in the body fluids of man, it is not possible from the observations of the present study to make any positive statements concerning the effectiveness of this compound in the treatment of tuberculosis in humans.

SUMMARY

Isonicotinic acid hydrazide (Nydrazid) was well tolerated in man in daily oral doses of 3.0 mg. per kg. for periods of four to sixteen weeks. The drug was rapidly absorbed and a high percentage was excreted in the urine during the twenty-four-hour period after ingestion. Appreciable concentrations of the drug were present in the cerebrospinal fluid within three hours of an oral dose of 2.0 to 3.0 mg. per kg. in patients without meningitis. In patients with tuberculous meningitis, the concentrations of drug in the cerebrospinal fluid after oral administration were substantially higher than the concentration necessary to inhibit *M. tuberculosis* H37Rv *in vitro*.

The administration of isonicotinic acid hydrazide for the four- to sixteen-week period on the 3.0 mg. per kg. daily dose was not associated with any manifestations of drug toxicity in any of the patients studied. It is probable that, with higher doses or more prolonged administration, evidences of toxicity may be encountered.

The period of study has been too short to permit any statements concerning the possible emergence of drug-resistant strains of *M. tuberculosis* in the patients who have received the drug.

Finally, though isonicotinic acid hydrazide possesses a high degree of activity against *M. tuberculosis* in animals and in the body fluids of man, it is not possible from the present observations to make any positive statement concerning the therapeutic value of this compound in the treatment of tuberculosis.

[7] Five patients with meningeal tuberculosis and an additional 3 with miliary tuberculosis are under study as part of a joint investigation with Dr. Charles M. Clark (U.S.P.H.S.) of the Indian Medical Service, Western Navajo Hospital, Tuba City, Arizona. The results of this study will be published subsequently by Dr. Clark in association with the present writers.

SUMARIO

La Absorción, Ezcreción y Toxicidad a Breve Plazo de la Hidracida del Acido Isonicotínico (Nydrazid) en el Hombre

La hidracida del àcido isonicotínico (Nydrazid) parece ser bien tolerada en el hombre a dosis de 3.0 mg. por kg. de peso. Se absorbe bien y es excretada en la orina durante un período de veinticuatro horas. Además, aparece en el líquido cefalorraquídeo en concentraciones moderadas en término de tres horas de la administración de una dosis oral de 2.0 a 3.0 mg. por kg. de peso. Durante los cuatro meses del período de observación no ha habido signos de toxicidad potencialmente grave.

RESUME

Absorption, excrétion et toxicité à brève échéance de l'hydrazide de l'acide isonicotinique (Nydrazid), chez l'homme

L'hydrazide de l'acide isonicotinique (Nydrazid) paraît bien tolérée chez l'homme à la dose de 3 mg. par kg. Elle est bien absorbée et est excrétée dans l'urine dans une période de vingt-quatre heures. De plus, elle apparaît en concentration modérée dans le liquide céphalorachidien dans les trois heures qui suivent l'administration d'une dose orale de 2 à 3 mg. par kg. Pendant les quatre mois de la période d'observation, aucun signe d'un potentiel de toxicité marquée n'a été observé.

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OBSERVATIONS ON THE ELECTROCARDIOGRAM IN SURGICALLY TREATED PULMONARY TUBERCULOSIS[1]

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INTRODUCTION

With the increasing use of major surgical procedures in the treatment of pulmonary tuberculosis, new problems arise in evaluating the patient as a surgical risk. The recent major advances in our knowledge and application of pulmonary function tests and in the early detection of chest tumors have increased the scope of the thoracic surgeon and have complicated the assessment of such surgically treated cases subsequently. The surgeon rightly demands certain assurances with regard to the patient's condition before he is able to undertake radical surgical measures. One of the major considerations in this respect is the state of the cardiovascular system. In addition, before a patient with pulmonary tuberculosis is offered major surgery, there is frequently a period during which he undergoes minor forms of collapse therapy. This fact, together with positional changes in the heart and mediastinum due to pulmonary fibrosis, pleural effusions, or empyema, makes this evaluation a difficult one. This is particularly true as the patient approaches the age when degenerative cardiac disease is prone to occur. One of the important factors to be considered in the evaluation of cardiac status is the electrocardiogram, and this paper will deal largely with that subject.

A great deal has been written on the electrocardiogram in pulmonary tuberculosis, both with and without collapse therapy. The inconsistency of the findings and conclusions leaves the problem unresolved. The situation has been further complicated by changes in electrocardiographic technique and by the use of a greater number of exploring electrodes.

METHOD

In order to clarify this problem in the writers' minds, a series of 68 cases admitted to the Vancouver Division of Tuberculosis Control was studied. Twelve lead electrocardiograms were taken before institution of collapse or surgical therapy and were repeated several weeks or more after the procedure was performed. The three standard, three augmented unipolar, and six unipolar precordial leads were employed. Each patient was examined clinically, and an inspection was made of the chest roentgenograms in order to determine, in general terms, the anatomic position of the heart and mediastinum.

The pulmonary tuberculosis ranged in severity from minimal to far advanced, with and without cavitation. In all cases the disease was classified as active. The types of therapy used in the 68 patients were as follows: thoracoplasty, 24

[1] Presented to the British Columbia Society of Internal Medicine at their fall meeting, October 6, 1951, in Vancouver, British Columbia.

cases; pneumoperitoneum, 12 cases; lobectomy, 11 cases; pneumothorax, 10 cases; pneumonectomy, 8 cases; and extrapleural pneumothorax, 3 cases. The electrocardiograms were classified broadly into three groups, *normal*, *borderline*, and *abnormal*, using the commonly accepted criteria of normality. The changes in electrical axis and precordial transitional zones were recorded.

TABLE 1

ELECTROCARDIOGRAPHIC ABNORMALITIES OBSERVED IN 11 OF 68 PATIENTS WITH SURGICALLY TREATED PULMONARY TUBERCULOSIS

PROCEDURE	CLASSIFICATION OF ELECTROCARDIOGRAMS				CHANGES IN ELECTRICAL AXIS			CHANGES IN TRANSITIONAL ZONE		
	Before		After		No change	More horizontal	More vertical	No change	Shift right	Shift left
Left lobectomy 4 cases	Normal	4	Normal	3	1	3		1	1	2
			Abnormal	1						
Right lobectomy 7 cases	Normal	7	Normal	7	5	2		2	2	3
Right pneumonectomy 2 cases	Normal	1	Normal	2	1	1		2		
	Abnormal	1								
Left pneumonectomy 6 cases	Normal	5	Normal	5	5	1		2	2	2
	Borderline	1	Abnormal	1						
Left extrapleural pneumothorax 2 cases	Normal	2	Normal	2	1	1		1		1
Right extrapleural pneumothorax 1 case	Normal	1	Normal	1	1				1	
Pneumoperitoneum 12 cases	Normal	11	Normal	12	9	1		4	3	5
	Borderline	1								
Right pneumothorax 6 cases	Normal	6	Normal	4	3	1	1	3	1	1
			Borderline	1						
			Abnormal	1						
Left pneumothorax 4 cases	Normal	4	Normal	3	1		1	1		1
			Abnormal	1						
Right thoracoplasty 10 cases	Normal	9	Normal	9	7		1	2	1	5
	Abnormal	1	Abnormal	1						
Left thoracoplasty 14 cases	Normal	12	Normal	12	8	3		1	2	8
	Abnormal	2	Abnormal	2						
Totals:	Normal	62	Normal	60						
	Abnormal	5	Abnormal	7						
	Borderline	1	Borderline	1						

RESULTS

A summary of the electrocardiographic abnormalities which were observed may be seen in table 1.

Left lobectomy: One of the 4 patients in the left lobectomy group, a 22-year-old man with a clinically normal heart and blood pressure of 165 systolic and 80 diastolic in mm. of mercury, had an abnormal electrocardiogram postoperatively.

There was a shift of the transitional zone to the left, and T_1 and T_{avl}, previously upright, became sharply inverted. No change in electrical axis or roentgenographic position of the heart was seen.

Right lobectomy: In all 7 cases in the right lobectomy group, the electrocardiograms obtained preoperatively and postoperatively were normal.

Right pneumonectomy: The electrocardiogram in one of the 2 cases in this group improved postoperatively. A flat T_1 and VT_6 became normally upright. There was no change in electrical axis or transitional zone, although the roentgenogram revealed a mediastinal shift to the right.

Left pneumonectomy: In this group of 6 cases, there was a change in one case. The abnormal preoperative electrocardiogram in this case of an 11-year-old boy showed right-axis deviation, depression of $ST_{1,2}$, and inversion of $VT_{1,2,3,4}$. Postoperatively, the roentgenogram revealed left mediastinal shift, and the electrical axis became more horizontal. The abnormal electrocardiogram showed a flat T_1, inverted T_{avl}, and depressed ST_{avl}.

Right and left extrapleural pneumothorax: There were no abnormal tracings in the 3 patients with right or left extrapleural pneumothorax.

Pneumoperitoneum: There was a minor change in only one of the 12 patients with pneumoperitoneum. Before institution of pneumoperitoneum in this case, T_1 was low, and the electrocardiogram was classified as borderline. T_1 became normally upright after surgery. There was no change in the electrical axis.

Right pneumothorax: Two of the 6 patients with right pneumothorax showed electrocardiographic changes after collapse therapy. In the first, the electrical axis shifted from an intermediate to a vertical position. There was no apparent positional change seen in the roentgenogram. The electrocardiogram, classified as borderline, showed deepening of S_1 and flattening of T_2. In the second case, the post-pneumothorax electrocardiogram showed a flat T_1, low $T_{2,3}$, and lowered precordial T-waves. There was no electrical axis shift. The tracing was classified as abnormal.

Left pneumothorax: Of the 4 patients with left pneumothorax, only one showed changes following institution of the collapse procedure. In this patient there was a lowering of voltage in the precordial leads and lead I; T_1 became almost flat. The electrical axis shifted from a semi-horizontal to a semi-vertical position. There was no obvious change on the roentgenograms in the position of the heart and mediastinum.

Right thoracoplasty: One patient of the total of 10 patients with right thoracoplasty had an abnormal electrocardiogram before and after thoracoplasty. The surgical procedure apparently did not affect the low voltage which was present.

Left thoracoplasty: There were electrocardiographic changes in 3 of the 14 patients with left thoracoplasty. In the first of these, the preoperative tracing was abnormal (inverted T_1); and after surgery, although there were minor changes, the tracing remained abnormal. In the second case, the preoperative tracing was abnormal (low T_1, flat T_2, inverted T_3, and T_{avf}). Following left thoracoplasty, the roentgenograms revealed a shift of the heart and mediastinum to the left. The electrical axis became more horizontal, and T_1, T_2, and T_{avf}

became upright. The electrocardiogram was interpreted as normal. In the third case, the electrocardiogram became abnormal following surgery, due to flattening of T_1.

DISCUSSION

The most important observation made in the present study is that the postoperative electrocardiograms were normal in the majority of cases. In 62 cases the electrocardiograms were classified as normal before surgery, and in 60 cases the tracings were considered to be normal after surgery. The conclusion is therefore justified that, although minor changes may occur in the electrocardiogram, these changes, by and large, are not such as to cause a reclassification of the tracings as abnormal. This is not in agreement with the conclusion reached by other writers, particularly Rauchwerger and Erskine (1) who report abnormal electrocardiograms in 30 per cent of their patients following major chest surgery.

In considering the effect of the different collapse and surgical procedures on the electrical axis of the electrocardiograms, again, in the majority of the present cases (42 cases), the electrical axis did not change. In contrast to these observations, one group (4) reports a more horizontal axis in all of their cases of left pneumonectomy and in the majority of their cases of thoracoplasty (left and right) and right pneumonectomy. Pollak (6) records a vertical axis in the majority of her patients following pneumoperitoneum, while Weinshel and his group (4) report a more horizontal axis in all of their cases. The same discrepancy was noted in several recent series concerned with the electrocardiographic changes following pneumothorax (2, 3, 4).

An analysis of the abnormal electrocardiograms may be seen in table 2. Of the total of 11 patients with electrocardiographic abnormalities at any time, 4 with normal tracings developed abnormal tracings after surgery (Cases 1, 5, 7, 10). One patient with a borderline tracing (Case 4) and 2 with definitely abnormal tracings (Cases 2 and 9) had normal electrocardiograms postoperatively. In 3 patients, abnormal tracings (Cases 3, 8, 11) remained abnormal following surgery. The most striking finding is that in each of the 4 cases in which abnormalities appeared following collapse therapy, the abnormality was essentially the same, namely, flattening or inversion of T_1, with or without low voltage. In each of these cases the electrical axis was semivertical or vertical. In one case in which the electrocardiogram became normal after left thoracoplasty (Case 9), the change was roughly the reverse of the above; that is, improvement in T_1 T_2, with the assumption of a more horizontal electrical axis.

Up to this point the present discussion has been concerned with recorded observations; now, in attempting to explain these changes, it is necessary to indulge in speculation. Some of the possible explanations are as follows:

I. Changes in myocardium due to:
   (a) collapse or surgical therapy
   (b) tuberculosis—"toxins," "bacterial invasion"
   (c) increased pressure in pulmonary circulation due to tuberculosis or pulmonary fibrosis

TABLE 2

ANALYSIS OF ABNORMAL AND BORDERLINE ELECTROCARDIOGRAMS

CASE NUMBER	AGE	SEX	PROCEDURE	CLASSIFICATION BEFORE SURGERY	ELECTROCARDIOGRAM AFTER SURGERY			OTHER FINDINGS
					Electrical axis	Transitional zone shift	Basis for classification as abnormal	
1	22	M	Left upper lobectomy	Normal	Vertical unchanged	To the left	T_1 and T_{av1} became sharply inverted	Blood pressure 165/80, no mediastinal shift Vertical heart
2	42	M	Right pneumonectomy	Abnormal	Intermediate unchanged	None	T_1 and VT_6 flat preoperatively, normally upright after surgery	Mediastinal shift to the right
3	11	M	Left pneumonectomy	Abnormal	More horizontal	To the left	T_1 flat, ST_{av1} depressed, T_{av1} inverted	Mediastinal shift to the left
4	27	F	Pneumoperitoneum	Borderline	Semi-vertical unchanged	To the left	T_1 low preoperatively, normal after pneumoperitoneum	
5	31	F	Right pneumothorax	Normal	Semi-vertical unchanged	None	T_1 flat, T_2, T_3 low	No mediastinal shift
6	32	F	Right pneumothorax	Normal	More vertical	To the left	Considered borderline, T_3 flat, S_1 deeper, R.A.D.	No roentgenographic change
7	29	F	Left pneumothorax	Normal	More vertical	To the left	Lowered voltage QRS_1 and V leads, T_1 low	No roentgenographic change
8	52	M	Right thoracoplasty	Abnormal			Low voltage, before and after surgery	
9	23	F	Left thoracoplasty	Abnormal	More horizontal	To the right	Low T_1, flat T_3, inverted T_{avf} became upright, normal ECG after surgery	Mediastinal shift to the left
10	25	F	Left thoracoplasty	Normal	Vertical		QRS_1 low voltage, T_1 flat	
11	56	F	Left thoracoplasty	Abnormal	Semi-horizontal unchanged	To the right	T_1 inverted before surgery, T_1 diphasic, and $VT_{5,6}$ flat after surgery	Blood pressure 150/90, mediastinal shift to the right

II. Myocardial damage unrelated to tuberculosis or collapse therapy:
  (a) coronary artery disease
  (b) severe anemia, et cetera
III. Myocardium undamaged, changes due to the interposition of air between the thoracic contents and the exploring electrodes
IV. Changes in the anatomic and electrical axis of the heart
V. Combination of the above.

Bjorkman (9), in his very comprehensive review of the subject, concludes that tuberculosis produces direct myocardial damage (and electrocardiographic changes) in a manner analogous to that produced by rheumatic fever. It is difficult, in the present study of patients with collapse therapy, to ascribe the changes observed to direct myocardial involvement by tuberculosis; nor is it possible to invoke changes in the conducting medium (air) as an explanation for the observations, since in 2 of the 4 cases there was no air present. Coronary artery disease is uncommon in this young age group (22 through 32 years); and anemia, severe enough to produce electrocardiographic changes, was not a factor.

Thus, two possibilities remain: (*1*) the electrocardiographic changes reflect pulmonary hypertension secondary to the underlying tuberculosis or to collapse therapy; or (*2*) the changes in the tracings reflect changes in the electrical and/or anatomic axis of the heart.

As yet there is little in the literature on pulmonary vascular pressure in pulmonary tuberculosis and collapse therapy. This aspect, therefore, requires further study. There was no evidence of acute or chronic right heart strain in any of the cases in the present study. The common factor in all of the patients was the vertical or semi-vertical position of the heart. The abnormalities found in 3 of the cases were essentially an exaggeration to a pathologic degree of the changes which occur in vertical hearts, i.e., lowering of T_1. In one case (Case 9), with the assumption of a more horizontal electrical axis and mediastinal shift to the left, the electrocardiogram became normal following surgery (T_1, T_2, T_{avf} became upright). Therefore, in the present small series, it may be inferred that the changes when observed were essentially positional in origin.

In evaluating patients, who have had collapse therapy, for further surgery, the electrocardiographic findings of low or flat T_1, in the presence of a clinically normal, vertical heart, should not be considered as indicating myocardial damage.

SUMMARY

With the increasing use of major surgical measures in the treatment of pulmonary disease, difficulties in the evaluation of the patient as a subsequent surgical risk have been stressed. The differentiation of electrocardiographic changes due to intrinsic cardiac disease from those due to surgery itself is important.

A series of 68 patients undergoing collapse therapy of various types for pulmonary tuberculosis was studied before and after surgery. Eleven patients had electrocardiographic changes following surgery. In 2 cases the electrocardiogram,

previously abnormal, became normal following surgery. In 3 cases the abnormal electrocardiograms remained abnormal. In only 5 cases was the surgical procedure followed by the first appearance of electrocardiographic abnormalities. In 4 of these cases the abnormalities were similar, i.e., low or flat T_1 associated with a semi-vertical or vertical electrical axis.

There were no consistent transitional zone or electrical axis changes in any of the groups studied.

The electrical position of the heart is particularly important in evaluating the electrocardiogram in patients who have had chest surgery. The value of multiple precordial and augmented unipolar limb leads in such cases has again been demonstrated. A plea is made for the use of twelve lead electrocardiograms in routine practice.

SUMARIO

Observaciones Electrocardiográficas en la Tuberculosis Pulmonar Tratada Quirúrgicamente

El empleo creciente de providencias de cirugía mayor en el tratamiento de las neumopatías acentúa las dificultades encontradas después al justipreciar al enfermo como riesgo quirúrgico. Por eso, resulta importante diferenciar las alteraciones electrocardiográficas debidas a cardiopatía intrínseca, de las creadas por la cirugía misma.

Una serie de 68 enfermos sometidos a colapsoterapia de varias formas por tuberculosis pulmonar fué estudiada antes y después de la operación. Once enfermos mostraron alteraciones electrocardiográficas consecutivamente a la operación. En 2 casos, el electrocardiograma, previamente anormal, se normalizó después de la cirugía. En 3, los electrocardiogramas anormales continuaron siéndolo. Sólo en 5 el procedimiento quirúrgico fué seguido de empeoramiento de electrocardiogramas normales. En 4 de estos casos la anomalía era semejante, o sea, hundimiento o aplanamiento de la T_1, unido a eje eléctrico semivertical o vertical.

En ninguno de los grupos estudiados hubo alteraciones constantes de la zona de transición o del eje eléctrico.

La posición eléctrica del corazón reviste en particular importancia al estudiar el electrocardiograma en los enfermos sometidos a la cirugía torácica. Queda de nuevo demostrado el valor de las derivaciones precordiales múltiples y unipolares aumentadas en los miembros, en esos casos. Abógase por el empleo de electrocardiogramas en doce derivaciones en la práctica corriente.

RESUME

Observations faites sur l'électrocardiogramme dans la tuberculose pulmonaire traitée chirurgicalement

En raison de l'augmentation croissante de procédés de grande chirurgie dans le traitement des affections pulmonaires, l'auteur souligne les difficultés rencontrées dans l'évaluation du malade, en tant que risque opératoire. Il est important de différencier les modifications de l'électrocardiogramme liées à une affection cardiaque intrinsèque, de celles dues à la chirurgie elle-même.

Une série de 68 malades soumise à diverses formes de collapsothérapie, pour traitement de la tuberculose pulmonaire, a été étudiée avant et après l'intervention chirurgicale. Onze malades présentaient des modifications de l'électrocardiogramme après l'opération; dans 2 cas, l'électrocardiogramme, qui était anormal, est devenu normal après l'intervention chirurgicale. Dans 3 cas les électrocardiogrammes anormaux sont restés anormaux. Dans 5 cas seulement l'intervention chirurgicale a été suivie de modifications pathologiques de l'électrocardiogramme normal. Dans 4 de ces cas les anomalies étaient semblables, c'est

à dire, que l'onde T était basse ou aplatie et associée à un axe électrique vertical ou semi-vertical.

Il n'existait de zone de transition constante ou de modification de l'axe électrique dans aucun des groupes étudiés.

La position électrique du coeur est particulièrement importante dans l'évaluation de l'électrocardiogramme chez les malades ayant été soumis à la chirurgie thoracique. La valeur des dérivations précordiales multiples et des dérivations unipolaires des membres, qui est augmentée dans les cas de ce genre, a été de nouveau démontrée. L'auteur insiste pour qu'il soit fait usage d'électrocardiogrammes à douze dérivations dans la pratique habituelle.

Acknowledgment

We wish to thank Dr. G. F. Kincade and the Department of Tuberculosis Control for the help given us in making this study possible.

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PREVALENCE OF PREVIOUSLY UNKNOWN TUBERCULOSIS IN PERSONS NOT PARTICIPATING IN MASS CHEST ROENTGENOGRAPHIC SURVEYS[1]

WM. T. PALCHANIS

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INTRODUCTION

Is the prevalence of unknown[2] tuberculosis different among participants and nonparticipants in mass surveys? The answer to this question which has concerned tuberculosis workers for a long time is still not known.

A recent contribution to the study of the question is that made by Comstock and Burke (1). They base their conclusions on a comparison of tuberculosis mortality rates. These workers found that:

> Tuberculosis mortality among the surveyed and nonsurveyed populations is not significantly different. Since part of the surveyed cases had treatment earlier in their disease than the nonsurveyed cases, their fatality may have been somewhat decreased. This suggests that the nonsurveyed population had proportionately fewer cases of tuberculosis than the surveyed; and the fact that the death rate was not more markedly decreased cannot be explained on the basis of undiscovered cases alone.

A recent study conducted at the Ohio State University Student Health Service demonstrates a much greater prevalence of previously unknown tuberculosis among unsurveyed students in that institution.

The number of previously unknown cases of active[3] and inactive[4] tuberculosis found in 100 nonparticipating students at Ohio State University approximated ten times the number of comparable cases found in 100 survey participants at the same university.

BACKGROUND AND METHOD OF STUDY

At the Ohio State University Student Health Service, mass chest roentgenographic and skin-testing surveys are conducted on new students at the beginning of each quarter. For various reasons, approximately 8 per cent of the new stu-

[1] From the Ohio State University Student Health Service and the Department of Preventive Medicine, Ohio State University Medical School, Columbus, Ohio.

[2] The designation "unknown" tuberculosis used in this paper refers to any form of pulmonary tuberculosis which was unsuspected, undiagnosed, unreported, and unknown to anyone previous to its discovery in this study.

[3] "Active" tuberculosis is used to designate characteristic pulmonary abnormalities observed on the chest roentgenogram associated with a positive cutaneous reaction to tuberculin, the presence of tubercle bacilli in the sputum or gastric contents, and recent symptoms.

[4] "Inactive" tuberculosis is used to designate a stable pulmonary lesion associated with a positive cutaneous reaction to tuberculin, the absence of tubercle bacilli in repeated examinations of the sputum and gastric contents, and no symptoms.

dents do not participate in the regularly scheduled surveys. Therefore, for purposes of this study, every effort was made during the past two years to test the cutaneous reaction to tuberculin and to obtain chest roentgenograms on as many as possible of this nonparticipating or "straggler" group.

The majority of this "straggler" group were brought for roentgenographic examination when the writer and his associates, seeing a student in the health service for other medical reasons, discovered that he or she had neglected to have a chest roentgenogram and skin test on entrance to the university. The remainder were reached by telephone, by postal card, or through their college offices, and were brought in more or less by compulsion.

OUTLINE OF EXAMINATION PROCEDURES

Participating or volunteer group: The initial roentgenographic examinations were made in the Ohio State Board of Health mobile units using the 70-millimeter film. The interpretations were made by examiners in the Division of Tuberculosis of the Ohio State Board of Health and rechecked by the writer. Follow-up studies of all suspects were made by the writer, using 4- by 5-inch stereo and 14- by 17-inch films.

Nonparticipating or "straggler" group: The 4- by 5-inch stereo film was used for the initial examination and the 14- by 17-inch film for the follow-up study of all suspects. All interpretations in this group were made by the writer.

Cutaneous reactions: At the time of the initial chest roentgenographic examination, skin tests were made which consisted of the intradermal injection of 0.1 cc. of tuberculin (PPD) containing 0.0001 mg., and 0.1 cc. of histoplasmin (Lilly Antigen) in 1:1,000 dilution. When suspicious roentgenographic densities were noted in a person with a negative cutaneous reaction to tuberculin on the initial test, the 0.005 mg. dose of tuberculin (PPD) was used for follow-up study.

OBSERVATIONS

For the purposes of comparison and for better appreciation of the significance of the findings obtained in this study, the data pertaining to both groups are given in table 1.

COMMENT

It is an obvious and very human failing that results in the fact that all college students and the population in general do not participate universally in voluntary mass roentgenographic programs when the opportunity affords.

The present study was made to gain some knowledge of the prevalence of previously unknown tuberculosis in a group of students at Ohio State University who failed to participate in regularly scheduled mass chest roentgenographic surveys.

In the participating or volunteer group of 10,232 students between the ages of eighteen and forty-two, there were 12 cases of previously unknown tuberculosis, 3 active and 9 inactive, or one person with previously unknown tuberculosis in every 852 persons examined (0.11 per cent).

In the nonparticipating or "straggler" group of 584 students between the

ages of eighteen and twenty-four, there were 7 cases of previously unknown tuberculosis, 2 active and 5 inactive, or one person with previously unknown tuberculosis in every 84 persons examined (1.19 per cent).

The percentage of previously unknown tuberculosis found in the nonparticipating or "straggler" group of students at Ohio State University approximates

TABLE 1

PREVALENCE OF PREVIOUSLY UNKNOWN* TUBERCULOSIS IN A GROUP OF 584 COLLEGE STUDENTS WHO DID NOT PARTICIPATE IN REGULARLY SCHEDULED CHEST ROENTGENOGRAPHIC SURVEYS COMPARED WITH A GROUP OF 10,232 COLLEGE STUDENTS WHO DID PARTICIPATE

GROUPS STUDIED	PARTICIPATING OR VOLUNTEER GROUP		NONPARTICIPATING OR STRAGGLER GROUP	
Period of study	June 1949 to June 1951		June 1949 to June 1951	
Ages	18 to 42		18 to 24	
	Total	Per cent	Total	Per cent
Number of students examined	10,232	100.00	584	100.00
Number of students with active‡ tuberculosis	3	0.029	2	0.34
Known†	0	0.000	0	0.000
Unknown*	3	0.029	2	0.34
Number of students with inactive§ tuberculosis	28	0.27	18	3.0
Known†	19	0.18	13	2.2
Unknown*	9	0.087	5	0.85
Total number of students with previously unknown* tuberculosis	12	0.11	7	1.19

* The designation "unknown" tuberculosis refers to any form of pulmonary tuberculosis which was unsuspected, undiagnosed, unreported, and unknown to anyone previous to its discovery in this study.

† The term "known" tuberculosis refers to any form of pulmonary tuberculosis which has been diagnosed, reported, treated, and known to the student and others prior to this study.

‡ "Active" tuberculosis is used to designate characteristic pulmonary abnormalities observed on the chest roentgenogram associated with a positive cutaneous reaction to tuberculin, the presence of tubercle bacilli in the sputum or gastric contents, and recent symptoms.

§ "Inactive" tuberculosis is used to designate a stable pulmonary lesion associated with a positive cutaneous reaction to tuberculin, the absence of tubercle bacilli in repeated examinations of the sputum and gastric contents, and no symptoms.

ten times the percentage found in the participating or volunteer group at the same university.

College and university students constitute a younger age group than the participants in the usual industrial or community survey. If these college student findings could be applied to other surveys, a startling possibility would present itself.

Assuming that 500,000 persons participated in a mass chest roentgenographic survey conducted in a city of 550,000 population, it could be postulated that the number of unknown cases of tuberculosis in the 50,000 persons who did not participate in the survey would approximate the number found in the 500,000 who did participate. It could thus be postulated that in such a study approximately one-half of the unknown cases of tuberculosis would remain undiscovered unless the entire group of 50,000 nonparticipants were included in the program.

SUMMARY

The incidence of previously unknown tuberculosis among Ohio State University students who did not participate in regularly scheduled mass chest roentgenographic surveys was relatively much greater than the incidence among the majority who did participate.

If these findings have generality, they should be taken into consideration by survey workers in interpretations of mass chest roentgenographic surveys.

SUMARIO

Frecuencia de la Tuberculosis Desconocida en las Personas Que No Participan en las Encuestas Radiotorácicas Colectivas

La incidencia de tuberculosis desconocida entre los estudiantes de la Universidad del Estado de Ohio que no participan en las encuestas radiográficas del tórax llevadas a cabo con regularidad en la colectividad, es relativamente mucho mayor que entre la mayoría de los que participan en aquellas.

Si esos hallazgos tienen aplicación general, deben tomarlos en cuenta los técnicos de las encuestas al interpretar los resultados de las mismas.

RESUME

Prédominance de la tuberculose inapparente chez les individus non inclus dans les programmes d'examens radiologiques collectifs du poumon

La fréquence de la tuberculose inapparente chez les étudiants de l'Université de l'Etat de Ohio, non inclus dans les investigations radiologiques périodiques, est relativement plus grande que celle qui est observée parmi la majorité incluse dans les investigations.

Si ces résultats se généralisaient, ils devraient être pris en considérations par les statisticiens dans l'interprétation des résultats fournis par les investigations radiologiques des collectivités.

Acknowledgment

The author is indebted to Dr. J. W. Wilce, Director, Ohio State University Health Service, for his invaluable guidance and assistance in the preparation of the manuscript; and to his associates for their cooperation and assistance in making this study possible.

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TRACTION DIVERTICULA OF THE ESOPHAGUS IN MIDDLE LOBE SYNDROME[1, 2]

HARRY L. KATZ

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INTRODUCTION

Middle lobe syndrome is characterized by a contraction of the middle lobe of the lung as a result of long-continued extrinsic pressure upon its bronchus by enlarged lymph nodes. In most instances the pathologic changes in these nodes represent residua of an old tuberculous infection. Many years may elapse before symptoms relative to the bronchial obstruction become manifest.

Brock (1) stressed the predisposition of the middle lobe bronchus to compression and obstruction by enlarged hilar lymph nodes because of its anatomic characteristics. The middle lobe bronchus is a long and narrow structure with thin walls and is surrounded by lymph nodes at its origin from the stem bronchus. These lymph nodes drain the middle lobe, the right lower lobe, and, by retrograde drainage, the right upper lobe. Graham, Burford, and Mayer (2), Paulson and Shaw (3), and Rubin and Rubin (4) have described cases of middle lobe syndrome and have presented the essential clinical, pathologic, and roentgenographic features in this disease entity.

The symptoms of middle lobe syndrome are those usually related to bronchial obstruction and bronchiectasis as manifested by intermittent hemoptyses, recurrent episodes of pneumonitis, chronic cough, chest pain, dyspnea, fatigability, and wheezing.

The main roentgenographic and pathologic changes in the syndrome consist of:

1) Partial or complete stenosis of the middle lobe bronchus or its segmental branches
2) Shrunken, consolidated, or bronchiectatic lobe
3) Atelectasis, fibrosis, and varying degrees of inflammatory changes in the pulmonary parenchyma
4) Calcified or enlarged hilar lymph nodes in close relation to the middle lobe bronchus.

Complete roentgenographic study for clear delineation of the nature and extent of the middle lobe involvement should include (in addition to the conventional postero-anterior view, right lateral and lordotic views) anteroposterior and right lateral tomograms, bronchography, and an esophagram. The bronchogram usually reveals complete or partial stenosis of the main or segmental branches of the middle lobe bronchus, bronchiectatic changes, irregularity or crowding of the bronchi consistent with fibrosis, and shrinkage of the lobe.

[1] From the Medical Service, Veterans Administration Hospital, Brooklyn, New York.

[2] Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are a result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

The fact that bronchopulmonary disease can produce displacement and retraction of the adjacent mediastinal structures is well known. Nevertheless, the association of middle lobe syndrome with localized retractions and diverticula of the esophagus has not been previously described in the literature. Advances in thoracic surgery have increased the indications for surgical intervention in pulmonary disease. Preoperative recognition of the presence of an associated esophageal diverticulum is essential, since it may be unexpectedly encountered at operation. The walls of the esophageal diverticulum may be firmly matted together with the diseased bronchus, middle lobe, and lymph nodes, thus exposing it to accidental surgical trauma unless its presence has been demonstrated prior to operation. In addition, if the diverticulum is large and symptom producing, consideration should be given to its correction when resection of the middle lobe is performed.

FIG. 1A. Fibrotic infiltration radiating from right hilum into middle lobe region; large lymph nodes and calcific densities are present within the right hilum.

B. Lordotic view; triangular and contracted middle lobe; the hilar nodes are well visualized.

The following five case reports serve to illustrate the association of traction diverticula of the mid-esophagus with chronic pulmonary disease of the middle lobe.

CASE REPORTS

Case 1. A 47-year-old white male was hospitalized with a ten-year history of chronic cough productive of a moderate amount of mucopurulent sputum, exertional dyspnea, and recurrent pneumonic episodes involving the right lung. The essential physical findings were moderate cyanosis of the mucous membranes, generalized rhonchi throughout both lung fields, and rales at the right base anteriorly. The chest was emphysematous in contour. Serial electrocardiographic tracings were consistent with right ventricular preponderance and cor pulmonale. The vital capacity was 50 per cent of normal; and the maximum breathing capacity, 40 liters per minute. There were no symptoms relating to the esophagus. Bronchoscopic examination revealed a narrow and chronically inflamed middle lobe bronchus; considerable tenacious mucoid sputum filled the middle and right lower bronchial tree. Chest roentgenograms (figures 1A and B) showed an irregular infiltration radiating from the lower right hilar region, a contracted middle lobe, hilar lymph node enlargement, and calcifications within these nodes. Bronchography (figure 1C) indicated saccular bron-

chiectasis and crowding of the bronchial segments within the shrunken and fibrotic middle lobe. An esophagram (figure 1D) revealed a saccular diverticulum arising from the right anterolateral wall of the mid-esophagus in close association with the hilar lymph nodes and middle lobe bronchus.

Case 2. A 63-year-old white male had complaints of cough productive of mucopurulent sputum, frequent respiratory infections, and several small hemoptyses of bright red blood for the past five years. The chest was emphysematous in contour, and expiratory rhonchi were audible bilaterally.

The electrocardiogram showed low voltage of the ventricular complexes in all leads. Pulmonary function tests showed a marked impairment in ventilation. There were no dysphagia or other symptoms suggesting disease of the esophagus. Bronchoscopic examination revealed hyperemia and granulation tissue in the right middle lobe bronchus. A chest

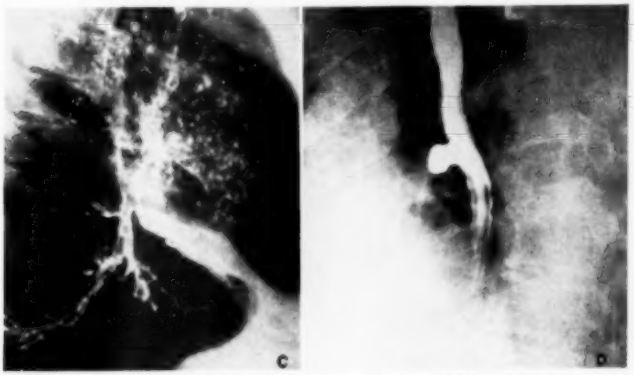

FIG. 1C. Bronchogram, right lateral view; saccular bronchiectasis and crowding of the bronchi within the contracted and dense middle lobe.

D. Esophagram; a saccular diverticulum in mid-esophagus in region of right hilar lymph nodes and middle lobe bronchus.

roentgenogram (figure 2A) showed an irregular fibrotic density adjacent to the right cardiac border and calcifications in the hilar regions. The lateral view (figure 2B) revealed a shrunken right middle lobe. Bronchography (figure 2C) indicated close grouping of the bronchial segments and blunt termination of the bronchi, as well as saccular and cylindrical bronchiectatic dilatations within the fibrotic and shrunken middle lobe. An esophagram (figure 2D) showed a small conical pouch-like diverticulum in the mid-esophagus at the level of the middle lobe bronchus.

Case 3. A 55-year-old white male was admitted to the hospital with complaints of dyspnea, productive cough of many years' duration, right chest pain, and recurrent episodes of pneumonia involving the right lung. He had mild postprandial substernal and epigastric discomfort for five years prior to admission. Examination revealed an emphysematous chest and crepitant rales at the right base. The electrocardiogram showed a pattern of left ventricular strain and an intraventricular conduction defect. Bronchoscopic examination showed the lumen of the middle lobe bronchus to be slitlike and the mucous membrane to be markedly indurated. A roentgenogram of the chest (figure 3A) showed considerable accentuation of the bronchial markings, multiple calcifications within the right hilum, and depression of the horizontal fissure. In the right lateral view (figure 3B), the middle lobe

appeared consolidated and triangular in shape. Bronchography (figure 3C) revealed the main middle lobe bronchus to be stenotic, and the segmental bronchi did not fill with the iodized oil. An esophagram (figure 3D) showed the presence of a large and triangular diver-

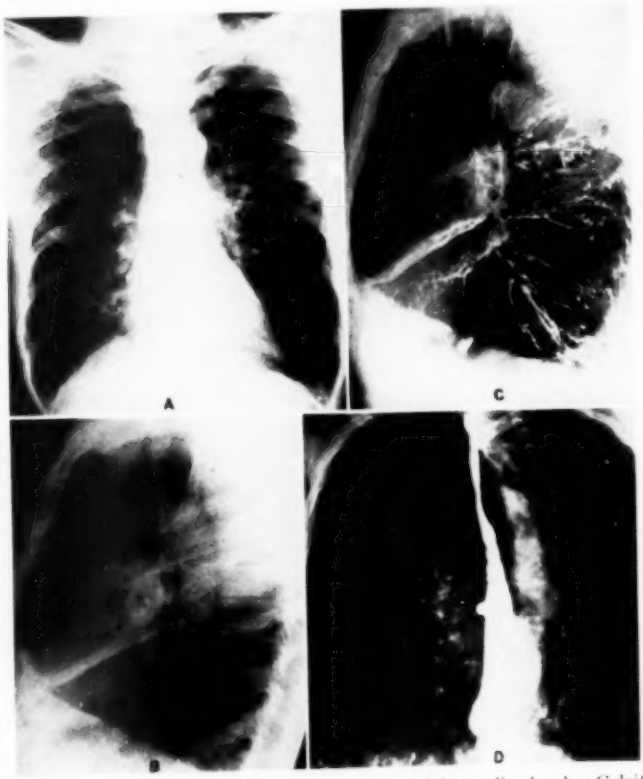

FIG. 2A. Patchy and fibrotic infiltration adjacent to right cardiac border. Calcifications are present within the hilar regions.

B. Right lateral view; the middle lobe is shrunken and atelectatic; the hilar lymph nodes are large and dense.

C. Bronchogram, right lateral view; close grouping of the segments of the middle lobe bronchi which terminate bluntly; saccular and cylindrical bronchiectasis is present.

D. Esophagram, a conical pouchlike diverticulum arises from lateral wall of mid-esophagus in region of middle lobe bronchus.

ticulum arising from the anterolateral wall of the mid-esophagus in close proximity to the calcified hilar lymph nodes and middle lobe bronchus.

Case 4. A 55-year-old white male gave a history of repeated pneumonic episodes involving the right lung, chronic cough, moderate dyspnea, and recurring hemoptyses of bright red

blood for the past ten years. Symptoms referable to the esophagus were absent. Physical examination revealed moderate clubbing of the fingers and toes. The thorax was emphy-

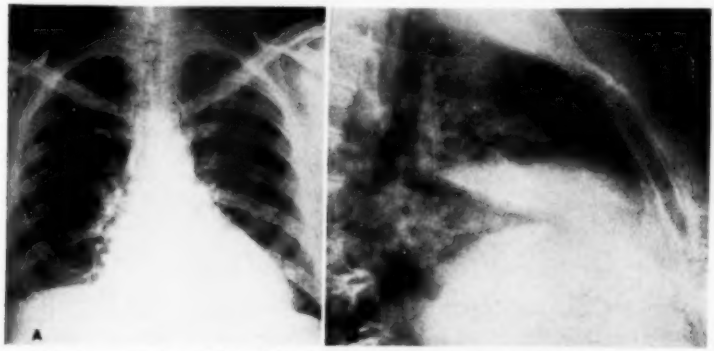

FIG. 3A. Accentuation of the right hilum, multiple calcific densities in hilar region, depression of the horizontal fissure.

B. Right lateral view; a dense and shrunken middle lobe.

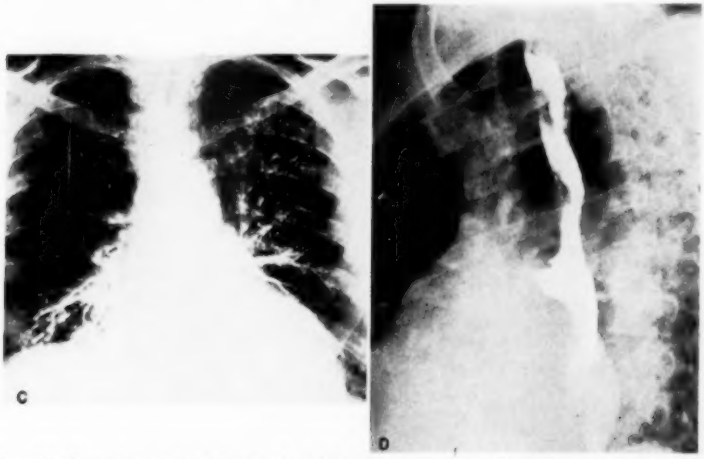

FIG. 3C. Bronchogram; stenosis of middle lobe bronchus with failure of iodized oil to fill the segmental bronchial branches.

D. Esophagram; a large diverticulum in mid-esophagus in close proximity to middle lobe bronchus.

sematous in contour; the diaphragmatic leaves were depressed and limited in motion. Expiratory rhonchi were audible at both bases. Bronchoscopy revealed the mucous membrane of the middle lobe bronchus to be hyperemic and indurated; tenacious mucoid exudate was present in the bronchial lumen. A mucosal biopsy showed chronic inflammatory tissue.

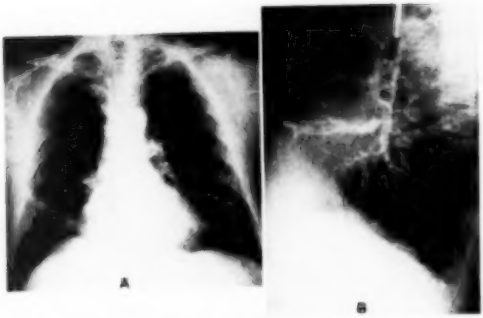

FIG. 4A. Right hilar adenopathy and an irregular fibrotic infiltration adjacent to right cardiac border.

B. Bronchography, right lateral view; crowding and irregularity of the segmental middle lobe bronchi.

FIG. 4C. Esophagram; saccular diverticulum in mid-esophagus; smaller diverticula are also present.

A chest roentgenogram (figure 4A) showed enlarged right hilar lymph nodes and an irregular fibrotic type of infiltration adjacent to the lower right cardiac border. Bronchography (figure 4B) revealed crowding and irregularity of the segmental bronchi of the middle lobe.

An esophagram (figure 4C) showed a large saccular diverticulum in the mid-esophagus in close proximity to the hilar nodes and middle lobe bronchus. Several small finger-like projections from the lateral wall of the esophagus were present proximally to the main diverticulum.

Case 5. A 61-year-old white male gave a history of right chest pain, chronic cough, wheezing, hemoptyses, and repeated pneumonic episodes involving the right lung. Examination

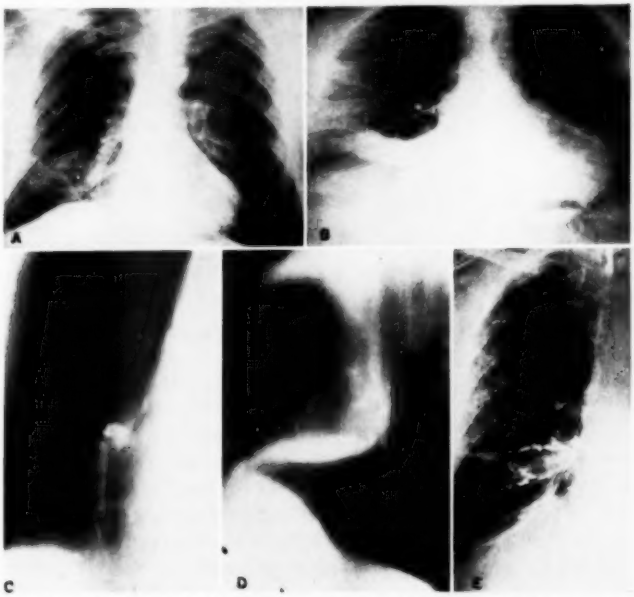

FIG. 5A. Irregular fibrotic infiltration in region of middle lobe; calcifications are present in both hila.

B. Lordotic view; a dense and shrunken middle lobe.

C. Tomogram; cluster of calcified lymph nodes surround the middle lobe bronchus.

D. Right lateral tomogram; dense triangular and sharply demarcated middle lobe.

E. Bronchogram; partial stenosis of middle lobe bronchus and complete occlusion of its lateral branch.

revealed asthmatic type of breath sounds in both lung fields, moderate cyanosis, and clubbing of fingers and toes.

The electrocardiographic tracing indicated low voltage and right ventricular preponderance. The vital capacity was 80 per cent of normal, and the maximum breathing capacity 54 liters per minute.

Bronchoscopic examination revealed a concentric narrowing of the orifice of the middle lobe bronchus; the mucous membrane was granular and edematous.

Roentgenographic studies of the chest (figures 5A, B, C, D) including postero-anterior, lordotic, and lateral tomographic views, showed a dense, atelectatic, and shrunken middle lobe and clusters of calcifications "saddling" the middle lobe bronchus. Bronchography

(figure 5E) revealed partial stenosis of the middle lobe bronchus with lack of filling of the lateral segmental branch. The esophagram (figure 5F) showed a saccular diverticulum in the mid-esophagus in close relation to the hilar calcifications and middle lobe bronchus.

FIG. 5F. Esophagram; a saccular diverticulum in mid-esophagus in close proximity to hilar nodes and middle lobe bronchus.

DISCUSSION

The incidence of esophageal diverticula in middle lobe syndrome is unknown; however, it is probably not rare. The 5 present cases were found in a series of 18 patients with middle lobe syndrome who had esophageal studies.

Vinson (5), Adams (6), and Lahey (7) have observed traction diverticula in the mid-esophagus associated with various inflammatory conditions involving the lungs and hilar regions, most commonly in patients with tuberculous mediastinal and bronchial lymph nodes. The diverticula were the result of an extension of the inflammatory reaction to the esophageal wall, formation of dense fibrous adhesions between the bronchial lymph nodes, peri-esophageal tissues, and the anterolateral wall of the esophagus. As a result of the shrinkage of the scar tissue, retraction and outpocketing of the esophageal wall occur.

Kragh (8), in a study of autopsied cases, found that traction diverticula in the mid-esophagus were in most instances secondary to chronic tuberculous lymph nodes in the hilar regions. These were most commonly found in individuals past the age of fifty years. Diverticula may also be secondary to caries of the spine, chronic pericarditis, and mediastinitis.

The diverticula associated with middle lobe disease are pouchlike extensions from the lumen of the esophagus. They are conical or triangular in shape, usually single, but may be multiple, and are composed of all layers of the esophageal wall. The neck of the diverticulum is wide and the apex usually points laterally and upwards, thus favoring its complete emptying.

Symptoms incident to the diverticulum are usually absent or minimal in degree. Retrosternal and epigastric discomfort attributable to the diverticulum was present in only one case in this study. Occasionally, however, angulation, obstruction, and ulceration of the esophagus may occur.

Sturtevant, Shapiro, and Wallace (9) cited instances of perforation of esophageal diverticula by calcified hilar lymph nodes. Jenkinson and Bate (10) and Melamed and Zimmerman (11) have described cases with perforation of the diverticulum into a bronchus with the formation of esophagobronchial fistula.

The presence of diverticula may be overlooked if the examination of the esophagus is not satisfactorily performed. The esophagus should be fully distended with a thick barium mixture and the roentgenograms should be taken with the patient in the recumbent position. In the standing position the barium may traverse the esophagus without entering the diverticulum, or it may remain in the pouch for a short time due to its rapid emptying.

Previous writers have emphasized the characteristic features of middle lobe disease including its symptomatology, roentgenographic, bronchoscopic, and bronchographic findings. The distortion and retraction of the mid-esophagus with formation of traction diverticula should be considered as part of the pattern of this disease entity.

SUMMARY

Five cases with traction diverticula of the mid-esophagus in association with chronic middle lobe disease have been presented.

Esophageal diverticula should be considered as part of the essential anatomic and roentgenographic pattern of middle lobe syndrome.

SUMARIO

Los Diverticulos de Tracción del Esófago en el Síndrome del Lóbulo Medio

Preséntanse 5 casos de divertículo de tracción del mesoesófago, unidos a enfermedad crónica del lóbulo medio.

Los divertículos esofágicos deben ser considerados como parte del patrón anatómico y radiográfico fundamental del síndrome del lóbulo medio.

RESUME

Diverticules de l'oesophage, par traction, dans le syndrome du lobe moyen

Présentation de cinq cas de diverticules de l'oesophage moyen, par traction, associés à une affection chronique du lobe moyen.

Les diverticules oesophagiens doivent être considérés comme faisant partie du type anatomique et radiologique fondamental du syndrome du lobe moyen.

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SPIROMETRIC STUDIES IN PNEUMOPERITONEUM[1, 2]

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INTRODUCTION

The therapeutic effect of pneumoperitoneum in pulmonary tuberculosis has been widely recognized in the last few years and its use has greatly increased. Despite this widespread use of the procedure, however, only a few studies on the effect of pneumoperitoneum on pulmonary function have been made. Bennett (1) found a drop in vital capacity immediately following the institution of pneumoperitoneum. The vital capacity was unchanged in 20 per cent and was increased in 25 per cent of those examined at various times following the established pneumoperitoneum. Steinlin (2) observed improvement in pulmonary functions when pneumoperitoneum was induced in the presence of an artificial phrenic paralysis. Banyai (3) summarizes his functional studies:

> My experience has revealed a reduction of the vital capacity in 76.9 per cent of the cases after the first treatment. The average decrease has been 12.5 per cent. Determination of the vital capacity during the course of pneumoperitoneum treatment has shown an average decrease of 14.7 per cent in the group under my observation.

Wright and co-workers (4) studied the effect of the fully established pneumoperitoneum in the erect and in the recumbent positions. They found a marked reduction of the functional residual air, residual air, and total lung volume, and a slight reduction of the maximum breathing capacity.

Functional effects of pneumoperitoneum used in the treatment of pulmonary emphysema were reported by Gaensler and Carter (5). Pulmonary function was improved as manifested by an increase in vital capacity, maximum breathing capacity, breathing reserve, and a reduction in residual air and ventilatory equivalent. Further functional improvement was found when one patient was restudied 94 days, and a second 167 days, after beginning treatment.

In the present investigation the pulmonary function of patients with pulmonary tuberculosis was studied before and after induction of pneumoperitoneum.

MATERIALS AND METHODS

The spirometric method used was closely similar to that described by Cournand, Richards, and Darling (6).

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[2] Sponsored by the Veterans Administration and published with approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect opinion or policy of the Veterans Administration.

Spirometry was always done under basal conditions. The patient was examined in the morning, without breakfast, resting on a stretcher.

The data which were determined were:

Oxygen intake per minute
Basal metabolic rate
Minute volume of respiration, that is, the amount of air ventilated in one minute
Tidal air
Respiratory rate
Ventilation equivalent for oxygen, that is, the amount of air in liters which a person ventilates in order to take up 100 cc. of oxygen.

The data were obtained under two conditions: first, when the patient was breathing air and, then, when he was breathing pure oxygen. Patients with significant degrees of anoxemia absorb more oxygen when breathing pure oxygen than when breathing air; an oxygen deficit exists. In the patients who underwent collapse therapy, the two sets of data were virtually the same and therefore only the figures determined under oxygen breathing will be presented. In addition, the following determinations were made:

Vital capacity
Reserve air
Complementary air
Maximum breathing capacity
The factor *maximum breathing capacity/minute volume*, which we call ventilatory reserve.

RESULTS

Pulmonary function was studied in 16 male patients with pulmonary tuberculosis before and after induction of pneumoperitoneum. No patient had phrenic paralysis at the time of receiving pneumoperitoneum. The first examination was performed a few days before collapse therapy was started; the subsequent examinations, one to thirteen months after pneumoperitoneum induction (table 1). Of the 16 patients, 10 had one examination after induction; 5 patients, after one month with pneumoperitoneum; 4 patients, after two months with pneumoperitoneum; and one patient, after six months with pneumoperitoneum. Six patients had serial determinations; 4 patients had two examinations after induction of pneumoperitoneum; and 2 patients had 3 examinations after induction of pneumoperitoneum.

Twenty-one of the 24 examinations were done one to six months after induction of the pneumoperitoneum.

In evaluating the functional results, it was convenient to divide the examinations into three groups: (A) examinations done one to three months after induction of pneumoperitoneum; (B) examinations done four to six months after induction of pneumoperitoneum; (C) serial determinations.

Group A (examined one to three months after induction of pneumoperitoneum): The spirometric findings before, and one to three months after, induction of pneumoperitoneum are shown in table 2. There were 13 patients in this group. In table 3 the mean, maximum, and minimum values before and after induction of pneumoperitoneum are given. In table 4 the frequency with which the changes occurred is indicated. Differences of less than 10 per cent between figures before and after induction of pneumoperitoneum are classed as unchanged.

Oxygen intake and basal metabolic rate decreased slightly in the mean but remained unchanged in the majority of the cases. The minute volume and tidal air showed a tendency to increase, the respiratory rate remained unchanged. The ventilation equivalent showed a slight tendency to increase. The vital capacity remained unchanged in the majority of the cases. Its mean decreased by only 6 per cent. The reserve air and complemental air tended to decrease. From table 5 it can be seen that the tidal air was a larger portion of the vital capacity after induction of pneumoperitoneum than before, whereas reserve air and complemental air were relatively smaller.

The maximum breathing capacity (table 3) showed a decrease of the mean by only 3 per cent. The factor *maximum breathing capacity/minute volume* showed a more definite tendency to decrease; its mean dropped by 12 per cent. In a few

TABLE 1

INTERVALS BETWEEN INDUCTION OF PNEUMOPERITONEUM AND RE-EXAMINATIONS

PATIENT NUMBER	NUMBER OF MONTHS BETWEEN PNEUMOPERITONEUM INDUCTION AND RE-EXAMINATION
1	5, 8, 13
2	4, 6, 9
3	6
4	2, 4
5	1
6	1
7	2
8	2
9	2
10	2
11	1
12	2, 6
13	2, 4
14	3, 6
15	1
16	1

No patient had phrenic paralysis at the time of receiving pneumoperitoneum

cases there was an increase in maximum breathing capacity and in maximum breathing capacity/minute volume.

Group B (examined four to six months after induction of pneumoperitoneum): The spirometric findings before, and four to six months after, induction of pneumoperitoneum are shown in table 6. There were 8 patients in this group. In table 7 are presented the mean, maximum, and minimum values before and after induction of pneumoperitoneum. In table 8 is indicated the frequency with which the changes occurred.

Oxygen intake and basal metabolic rate decreased slightly in the mean but remained unchanged in the majority of the cases. The minute volume showed a tendency to increase. As the respiratory rate decreased at the same time, the

TABLE 2

GROUP A (EXAMINED ONE TO THREE MONTHS AFTER INDUCTION OF PNEUMOPERITONEUM).
SPIROMETRY BEFORE AND AFTER INDUCTION OF PNEUMOPERITONEUM

Patient number	4	5	6	7	8	9	10	11	12	13	14	15	16
Age	55	39	27	57	25	22	21	56	29	43	35	48	25
Interval before and after, months	2	1	1	2	2	2	2	1	2	2	3	1	1
Oxygen intake in cc.													
before	199	231	200	271	251	231	255	256	263	227	336	314	222
after	235	204	200	259	229	236	251	196	225	213	232	300	225
Basal metabolic rate in per cent													
before	−20	−9	−17	+21	+3	−11	−10	+22	+8	+1	+32	+30	−5
after	−4	−17	−17	+14	−5	−9	−11	−6	−5	−1	−8	+25	−3
Minute volume in cc.													
before	5,930	5,490	5,010	6,640	5,230	4,630	6,490	8,090	5,600	6,270	4,740	10,620	4,840
after	8,460	5,010	5,610	9,700	6,970	4,630	5,770	8,250	5,930	5,930	6,210	12,800	5,930
Tidal air in cc.													
before	494	549	417	369	523	771	590	404	1,120	418	431	708	691
after	940	501	510	462	498	926	481	434	1,186	371	776	914	847
Respirations per minute													
before	12	10	12	18	10	6	11	20	5	15	11	15	7
after	9	10	11	21	14	5	12	19	5	16	8	14	7
Ventilation equivalent in liters													
before	2.5	2.1	2.2	2.1	1.8	1.7	2.4	2.7	1.8	2.4	1.2	2.9	1.9
after	3.1	2.1	2.4	3.2	2.6	1.7	2.0	3.6	2.5	2.4	2.3	3.7	2.3
Vital capacity in cc.													
before	3,220	2,920	2,740	2,880	2,470	2,670	5,270	2,860	1,910	2,900	3,650	4,150	3,030
after	3,030	2,490	3,010	2,740	2,430	2,280	4,750	2,260	1,840	2,800	3,710	4,040	2,880
Reserve air in cc.													
before	390	829	456	539	808	746	1,077	1,036	622	1,244	1,182	912	684
after	456	684	601	456	643	394	601	1,182	477	1,037	1,057	518	498

Complementary air in cc.													
before	2,336	1,542	1,867	1,972	1,139	1,153	3,603	1,420	168	1,238	2,037	2,530	1,655
after	1,634	1,305	1,899	1,822	1,289	960	3,668	644	177	1,392	1,877	2,608	1,535
Maximum breathing capacity in liters													
before	94.3	93.4	98.0	87.1	94.3	103.4	153.6	43.9	62.6	52.7	130.6	78.2	101.6
after	83.5	95.2	119.7	86.2	73.5	87.1	145.6	27.2	45.8	65.9	173.1	71.4	80.9
Maximum breathing capacity													
Minute volume													
before	15.9	17.0	19.6	13.1	18.0	22.3	22.1	5.4	11.2	8.4	27.7	7.4	21.0
after	9.9	19.0	21.3	8.9	10.6	18.8	25.3	3.3	7.7	11.1	27.8	5.6	13.7

average tidal air increased (by 17 per cent). The ventilation equivalent showed a slight tendency to increase. The vital capacity remained unchanged in the majority of the cases; it increased in one case. Its mean remained practically the same. The reserve air tended to decrease; the complemental air showed a tendency

TABLE 3

GROUP A (EXAMINED ONE TO THREE MONTHS AFTER INDUCTION OF PNEUMOPERITONEUM). SPIROMETRY BEFORE AND AFTER INDUCTION OF PNEUMOPERITONEUM

Mean, Maximum, and Minimum Data

	MEAN		HIGHEST		LOWEST	
	Before	After	Before	After	Before	After
Oxygen intake in cc.	250	231	336	300	199	196
Basal metabolic rate in per cent.	+4	−4	+32	+25	−20	−17
Minute volume in cc.	6,120	7,020	10,620	12,800	4,630	4,630
Tidal air in cc.	510	585	1,120	1,186	369	434
Respirations per minute	12	12	20	21	5	5
Ventilation equivalent in liters	2.1	2.6	2.9	3.7	1.2	1.7
Vital capacity in cc.	3,130	2,940	5,270	4,750	1,910	1,840
Reserve air in cc.	810	662	1,244	1,182	390	394
Complemental air in cc.	1,810	1,693	3,361	3,668	168	177
Maximum breathing capacity in liters	91.8	88.8	153.6	173.1	52.7	27.2
Maximum breathing capacity / Minute volume	16.1	14.1	27.7	27.8	5.4	3.3

TABLE 4

GROUP A (EXAMINED ONE TO THREE MONTHS AFTER INDUCTION OF PNEUMOPERITONEUM). CHANGES IN TOTAL LUNG FUNCTION CAUSED BY PNEUMOPERITONEUM

	INCREASED	UNCHANGED	DECREASED
Oxygen intake	1	8	4
Basal metabolic rate	1	9	3
Minute volume	7	5	1
Tidal air	7	4	2
Respirations per minute	2	8	3
Ventilation equivalent	8	4	1
Vital capacity	0	9	4
Reserve air	3	1	9
Complemental air	3	6	4
Maximum breathing capacity	3	4	6
Maximum breathing capacity / Minute volume	2	2	9

to increase. The data presented in table 9 reveal that tidal air and complemental air increased slightly in their relation to vital capacity, whereas the reserve air showed a relative decrease.

The maximum breathing capacity (table 7) remained unchanged. With the maximum breathing capacity remaining unchanged and the minute volume

slightly increasing, there was a tendency of the ventilatory reserve to decrease (8 per cent) in the mean.

Group C (serial determinations): Repeated spirometric examinations at various intervals after induction of pneumoperitoneum were done in 6 patients. The findings are presented in table 10.

A slight decrease of oxygen intake and basal metabolic rate was noted in most examinations after induction of the pneumoperitoneum. The minute volume, which increased first, later showed a tendency to decrease and to approach the original value. The respiratory rate tended to decrease, and the tidal air to increase, consequently. The ventilation equivalent was increased in the earlier examinations, less so in the later ones. The vital capacity did not show any significant changes. The reserve air decreased and remained diminished. The complementary air showed irregular increases and decreases.

TABLE 5

GROUP A (EXAMINED ONE TO THREE MONTHS AFTER INDUCTION OF PNEUMOPERITONEUM). MEANS OF SUBDIVISIONS OF VITAL CAPACITY IN PER CENT OF VITAL CAPACITY BEFORE AND AFTER INDUCTION OF PNEUMOPERITONEUM

	TIDAL AIR	RESERVE AIR	COMPLEMENTAL AIR
Mean			
before	20	27	53
after	25	24	51
Highest			
before	59	43	73
after	65	52	77
Lowest			
before	12	12	9
after	10	13	9

The maximum breathing capacity did not change in any characteristic way; there were irregular increases and decreases. The ventilatory reserve usually dropped first; later it tended to reach the original value.

DISCUSSION

Pneumoperitoneum used in the treatment of pulmonary tuberculosis has very little effect on pulmonary functions, as shown by the above studies.

Oxygen intake and basal metabolic rate decreased slightly, similar to the findings in other forms of collapse therapy (7). Minute volume and tidal air showed a tendency to increase in the first few months but returned later to the pretreatment levels. The ventilation equivalent increased slightly as it does under pneumothorax (8) and thoracoplasty (9).

The vital capacity did not show any significant changes. This finding contrasts with the results of similar studies in pneumothorax and thoracoplasty (8, 9) in which definite decreases of the vital capacity were found. There was an increase of tidal air in percentage of vital capacity. This was a consequence of an absolute

increase of the tidal air in the presence of a decrease of reserve air and complemental air.

TABLE 6

GROUP B (EXAMINED FOUR TO SIX MONTHS AFTER INDUCTION OF PNEUMOPERITONEUM). SPIROMETRY BEFORE AND AFTER INDUCTION OF PNEUMOPERITONEUM

Patient number	1	2	2	3	4	12	13	14
Age	26	31	31	57	55	29	43	35
Interval before and after, months	5	4	6	6	4	6	4	6
Oxygen intake in cc.								
before	209	234	234	216	199	263	227	336
after	207	228	218	224	193	218	222	231
Basal metabolic rate in per cent								
before	−6	+1	+1	+7	−20	+8	+1	+32
after	−2	−3	−12	+10	−19	−8	+5	−6
Minute volume in cc.								
before	4,830	5,710	5,710	7,280	5,930	5,600	6,270	4,740
after	5,660	6,640	5,550	7,080	6,870	4,970	6,150	5,280
Tidal air in cc.								
before	372	336	336	364	494	1120	419	431
after	472	443	397	416	687	994	440	660
Respirations per minute								
before	13	17	17	20	12	5	15	11
after	12	15	14	17	10	5	14	8
Ventilation equivalent in liters								
before	2.0	2.3	2.3	2.9	2.5	1.8	2.4	1.2
after	2.3	2.6	2.2	2.6	3.1	2.0	2.4	2.0
Vital capacity in cc.								
before	2,090	2,470	2,470	2,570	3,220	1,910	2,900	3,650
after	2,090	2,550	2,510	2,990	3,030	1,870	2,650	3,710
Reserve air in cc.								
before	498	705	705	456	390	622	1,244	1,182
after	332	498	435	643	435	560	1,057	995
Complementary air in cc.								
before	1,220	1,429	1,429	1,750	2,336	168	1,237	2,037
after	1,286	1,609	1,678	1,931	1,908	316	1,156	2,055
Maximum breathing capacity in liters								
before	58.1	80.4	80.4	74.7	94.3	62.6	52.7	130.6
after	59.3	76.2	78.2	70.7	87.9	54.9	46.7	149.7
Maximum breathing capacity / Minute volume								
before	12.1	14.1	14.1	10.3	15.9	11.2	8.4	27.7
after	9.6	11.4	14.1	10.0	12.8	11.0	7.6	28.4

The maximum breathing capacity remained unchanged. The ventilatory reserve (maximum breathing capacity/minute volume) dropped first (but less than under pneumothorax and thoracoplasty (8, 9)) and gradually returned to the original values.

These observations show that pneumoperitoneum does not interfere with pulmonary functions under basal conditions. The functional changes are much less

TABLE 7

GROUP B (EXAMINED FOUR TO SIX MONTHS AFTER INDUCTION OF PNEUMOPERITONEUM). SPIROMETRY BEFORE AND AFTER INDUCTION OF PNEUMOPERITONEUM

Mean, Maximum, and Minimum Data

	MEAN		HIGHEST		LOWEST	
	Before	After	Before	After	Before	After
Oxygen intake in cc.	240	217	336	231	199	193
Basal metabolic rate in per cent.	+3	−6	+32	+10	−20	−19
Minute volume in cc.	5,760	6,020	7,280	7,080	4,740	4,970
Tidal air in cc.	411	502	1,120	994	336	416
Respirations per minute	14	12	20	17	5	5
Ventilation equivalent in liters	2.2	2.4	2.9	3.1	1.2	2.0
Vital capacity in cc.	2,660	2,680	3,650	3,710	1,910	1,870
Reserve air in cc.	715	619	1,244	1,057	390	332
Complemental air in cc.	1,534	1,559	2,336	2,055	168	316
Maximum breathing capacity in liters	79.2	78.0	130.6	149.7	52.7	54.9
Maximum breathing capacity / Minute volume	14.2	13.1	27.7	28.4	8.4	7.6

TABLE 8

GROUP B (EXAMINED FOUR TO SIX MONTHS AFTER INDUCTION OF PNEUMOPERITONEUM). CHANGES IN TOTAL LUNG FUNCTION CAUSED BY PNEUMOPERITONEUM

	INCREASED	UNCHANGED	DECREASED
Oxygen intake	0	6	2
Basal metabolic rate	0	5	3
Minute volume	4	3	1
Tidal air	6	1	1
Respirations per minute	0	3	5
Ventilation equivalent	5	3	0
Vital capacity	1	7	0
Reserve air	2	1	5
Complemental air	3	4	1
Maximum breathing capacity	1	5	2
Maximum breathing capacity / Minute volume	0	5	3

TABLE 9

GROUP B (EXAMINED FOUR TO SIX MONTHS AFTER INDUCTION OF PNEUMOPERITONEUM). MEANS OF SUBDIVISIONS OF VITAL CAPACITY IN PER CENT OF VITAL CAPACITY BEFORE AND AFTER INDUCTION OF PNEUMOPERITONEUM

	TIDAL AIR	RESERVE AIR	COMPLEMENTAL AIR
Mean			
before	21	28	51
after	23	23	54
Highest			
before	59	43	73
after	53	40	67
Lowest			
before	12	12	8
after	14	14	17

TABLE 10

GROUP C (SERIAL DETERMINATIONS). SPIROMETRY BEFORE AND AFTER INDUCTION OF PNEUMOPERITONEUM

Patient number	1				2				4			12			13			14		
Months after treatment	0	5	8	13	0	4	6	9	0	2	4	0	2	6	0	2	4	0	3	6
Oxygen intake in cc	209	207	212	196	234	222	218	233	199	235	193	263	225	218	227	213	222	336	232	231
Basal metabolic rate in per cent	−6	−2	0	−10	+1	−3	−12	−7	−20	−4	−19	+8	−5	−8	+1	−1	+5	+32	−8	−6
Minute volume in cc	4,830	5,660	5,010	4,690	5,710	6,640	5,550	5,710	5,930	8,460	6,870	5,600	5,930	4,970	6,270	5,930	6,150	4,740	6,210	5,280
Tidal air in cc	372	472	386	361	336	443	397	476	494	940	687	1,120	1,186	994	419	371	440	431	778	660
Respirations per minute	13	12	13	13	17	15	14	12	12	9	10	5	5	5	15	16	14	11	8	8
Ventilation equivalent in liters	2.0	2.3	2.0	2.1	2.3	2.6	2.2	2.1	2.5	3.1	3.1	1.8	2.5	2.0	2.4	2.4	2.4	1.2	2.3	2.0
Vital capacity in cc	2,090	2,090	2,140	2,260	2,470	2,550	2,510	2,490	3,220	3,030	3,030	1,910	1,840	1,870	2,900	2,800	2,650	3,650	3,710	3,710
Reserve air in cc	498	332	477	518	705	498	435	394	390	456	435	622	477	560	1,244	1,037	1,057	1,182	1,057	995
Complementary air in cc.	1,220	1,286	1,277	1,381	1,429	1,609	1,678	1,620	2,336	1,634	1,908	168	187	316	1,237	1,392	1,156	2,037	1,875	2,055
Maximum breathing capacity in liters	58.1	59.3	62.6	51.2	80.4	76.2	78.2	65.3	94.3	83.5	87.9	62.6	45.8	54.9	52.7	65.9	46.7	130.6	173.1	149.7
Maximum breathing capacity / Minute volume	12.1	9.6	12.5	10.9	14.1	11.4	14.1	11.4	15.9	9.9	12.8	11.2	7.7	11.9	8.4	11.1	7.6	27.7	27.8	28.4

extensive than the changes caused by artificial pneumothorax or thoracoplasty. Wright, Place, and Princi (4) point out that the slight reduction of the maximum breathing capacity "that results from pneumoperitoneum, together with the fact that it is a reversible form of treatment, makes it possible to use this form of collapse therapy in cases of severe respiratory crippling." Clinical experience has shown that pneumoperitoneum can be given to patients who, because of poor pulmonary functions, could not tolerate a pneumothorax.

SUMMARY

Pulmonary function studies were done in 16 patients with pulmonary tuberculosis before, and one to thirteen months after, induction of pneumoperitoneum.

One to three months after pneumoperitoneum induction, the mean values showed the following behavior: There was a slight decrease of the oxygen intake and basal metabolic rate; a slight increase of minute volume and tidal air, with an unchanged respiratory rate; a slight increase of the ventilation equivalent; a slight decrease of vital capacity, reserve air, and complemental air; a slight decrease of the maximum breathing capacity; and a decrease of the ventilatory reserve.

Four to six months after pneumoperitoneum induction, the changes were less marked than they were one to three months after induction. Repeated studies done in the same patients showed that all values tended to return to pretreatment levels.

The effects of artificial pneumoperitoneum on lung functions are very small, much less extensive than the effects of pneumothorax and thoracoplasty.

SUMARIO

Estudios Espirométricos en el Neumoperitoneo

En 16 tuberculosos pulmonares ejecutáronse estudios de la función pulmonar antes y de uno a trece meses después de inducir el neumoperitoneo.

De uno a tres meses después de la inducción del neumoperitoneo, las cifras medias revelaron lo siguiente. Había ligera disminución de la inspiración de oxígeno y del coeficiente del metabolismo basal; leve aumento del volumen de aire por minuto y del aire corriente, sin cambio de la frecuencia respiratoria; leve aumento del equivalente ventilatorio; leve disminución de la capacidad vital, del aire de reserva y del aire complementario; leve disminución de la capacidad respiratoria máxima, y disminución de la reserva respiratoria.

De cuatro a seis meses después de la inducción del neumoperitoneo, las alteraciones eran menos pronunciadas que de uno a tres meses después de la misma. Estudios repetidos verificados en los mismos enfermos mostraron que todos los valores tendían a volver a las cifras pretratamiento.

Los efectos del neumoperitoneo terapéutico sobre las funciones pulmonares son pequeñísimos, mucho menos extensos que los del neumotórax o de la toracoplastia.

RESUME

Études spirométriques dans le pneumopéritoine

Des recherches sur la fonction pulmonaire ont été faites chez 16 malades atteints de tuberculose pulmonaire, d'abord avant la création d'un pneumopéritoine puis ensuite de un à treize mois après.

Un à trois mois après la création du pneumopéritoine, les valeurs moyennes ont présenté les aspects suivants: légère diminution de la consommation d'oxygène et du taux du métabolisme basal; légère augmentation du volume d'air par minute et de l'air de ventilation sans modification du taux respiratoire; une légère augmentation de l'équivalent de ventilation; une légère diminution de la capacité vitale, air de réserve et air complémentaire; une légère diminution de la capacité respiratoire maxima et une diminution de la réserve de ventilation.

Quatre à six mois après l'instauration du pneumopéritoine, les changements étaient moins marqués que un à trois mois après la création du pneumopéritoine. Des études répétées, effectuées chez les mêmes malades, montraient que toutes les valeurs tendent à retourner au niveau précédant le traitement.

Les effets du pneumopéritoine artificiel sur la fonction pulmonaire sont très faibles, beaucoup moins étendus que les effets produits par le pneumothorax et la thoracoplastie.

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CORRELATION OF BIOLOGIC PROPERTIES OF STRAINS OF MYCOBACTERIUM WITH THEIR INFRARED SPECTRUMS[1, 2]

II. The Differentiation of Two Strains, H37Rv and H37Ra, of *M. Tuberculosis* by Means of Their Infrared Spectrums

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INTRODUCTION

In a preceding paper (1) it was demonstrated that with sufficiently standardized procedures it was possible to obtain fractions of *M. tuberculosis* of such constant chemical content that the infrared spectrums were consistent for any given fraction. Moreover, the various fractions differed enough from each other to permit spectroscopic differentiation of the fractions.

The present investigation has as its purpose the establishment of the spectroscopic differentiation of two strains, namely, H37Rv and H37Ra.

MATERIALS AND METHODS

The same standardized method of inoculation, growth, and harvesting of the cultures was used as in the earlier work, but the fractionations were somewhat different. The procedures indicated by the accompanying flow diagram were employed. The fractionation was carried out in triplicate, starting with three approximately equal masses of bacilli. The harvested bacteria from seven culture flasks were combined to give the material for each of the triplicate sets of fractions. When the extractions indicated in the diagram were made, four fractions were obtained in triplicate from each of the two strains, namely, those numbered 2, 4, 6, and 8. The infrared spectrums of these triplicate fractions will show how well the H37Rv and H37Ra fractions have been reproduced. From the four different fractions it may be possible to choose one which will serve better than the others for spectroscopic differentiation of the H37Rv from the H37Ra strain. These two strains[3] were chosen since the avirulent strain H37Ra was originally derived from the virulent H37Rv strain, and differences in composition, as indicated by the infrared spectrums, could possibly be related to this biologic variation.

These fractionations have been carried out in their entirety on two different occasions approximately six weeks apart. The first time the fractionation was done there was only enough culture material for duplicate samples of H37Rv and for just one sample of each of the H37Ra fractions. As the flow diagram indicates, the harvested bacilli were allowed to stand in ethanol for forty-eight hours. The cell residue was separated by filtration on a Buchner funnel and was then washed with fresh ethanol. This residue was then extracted for twenty-four hours at 25°C. with a mixture of equal parts of methanol and chloroform. The residue was separated by filtration and rejected. The soluble fraction was divided into two parts. One part, upon evaporating the extracting mixture was labeled fraction No. 2. The other part was allowed to stand for twenty-four hours at 5°C. A precipitate formed which was filtered out and designated insoluble fraction No. 4. Then the solution was cooled

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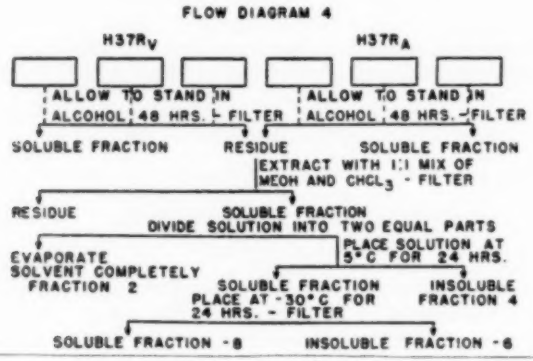
[1] From the Departments of Bacteriology and Physics, University of Michigan, Ann Arbor, Michigan.

[2] Aided by a grant from the Veterans Administration.

[3] Obtained through the courtesy of Mr. William Steenken, Jr., of the Trudeau Laboratories, Trudeau, New York.


FLOW DIAGRAM 4
H37Rv
H37RA
ALLOW TO STAND IN ALCOHOL 48 HRS. - FILTER
ALLOW TO STAND IN ALCOHOL 48 HRS. - FILTER
SOLUBLE FRACTION
RESIDUE
SOLUBLE FRACTION
EXTRACT WITH 1:1 MIX OF MEOH AND CHCL3 - FILTER
RESIDUE
SOLUBLE FRACTION
DIVIDE SOLUTION INTO TWO EQUAL PARTS
EVAPORATE SOLVENT COMPLETELY
FRACTION 2
PLACE SOLUTION AT 5°C FOR 24 HRS.
SOLUBLE FRACTION
PLACE AT -30°C FOR 24 HRS. - FILTER
INSOLUBLE FRACTION 4
SOLUBLE FRACTION - 8
INSOLUBLE FRACTION - 6

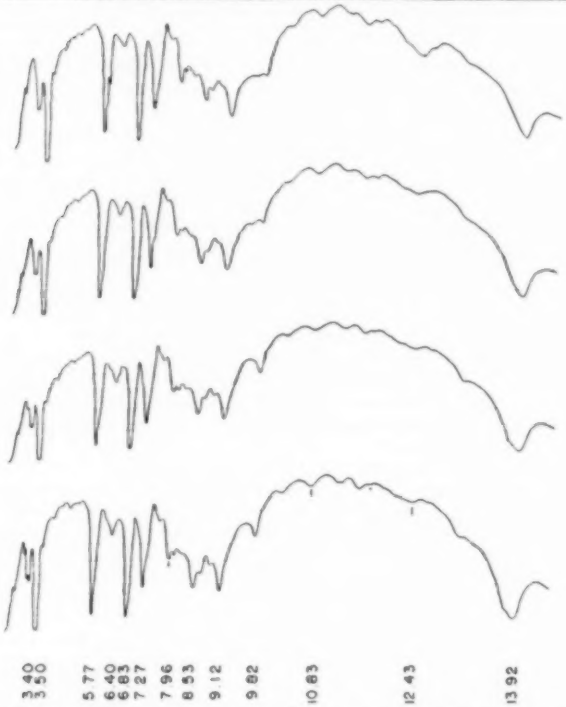


PLATE 1. These spectrums are of the No. 4 fraction of the H37Rv strain. The first two are from cultures harvested March 3, 1951; the last two are from cultures harvested May 11, 1951.

to −30°C. for twenty-four hours. A second insoluble precipitate formed which was separated by filtration and called insoluble fraction No. 6. The remaining soluble material, fraction No. 8, was deposited upon evaporating the solvent.

RESULTS

While all four fractions showed a satisfactory degree of reproducibility and all permitted a spectroscopic differentiation of the H37Rv strain from H37Ra, one fraction proved better adapted for the purpose than the others and therefore only spectrums of this fraction, the No. 4 fraction, will be reproduced.

The spectrums of the No. 4 fractions of H37Rv are to be found on plate 1. The top two spectrums are of the fractions prepared six weeks previous to the

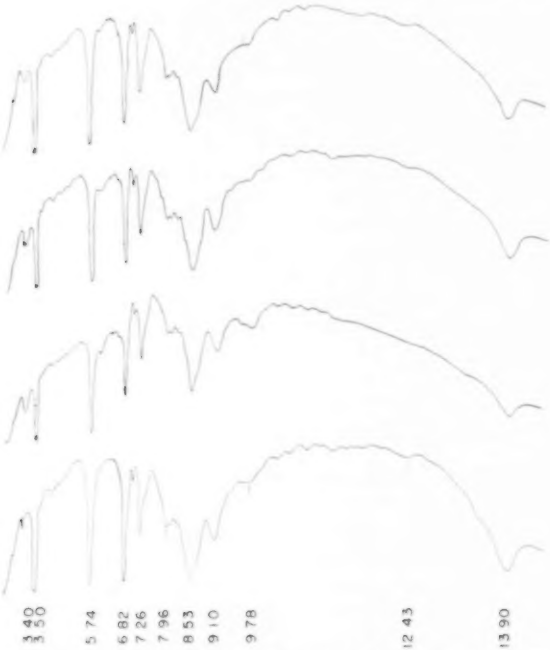


PLATE 2. These spectrums are of the No. 4 fraction of the H37Ra strain. The first one is from cultures harvested March 3, 1951; the last three are from cultures harvested May 11, 1951.

fractions represented by the two lower spectrums. The third spectrum of the latter set is not reproduced but it is identical to the other two. An inspection of plate 1 shows that this fraction of H37Rv has been reproduced with a very high degree of accuracy. This is true, not only between the fractions prepared at the same time, but also between those prepared on different dates. The largest variation in the spectrums of H37Rv is in the somewhat greater intensity of the 12.44μ band of the top spectrum. Also the band at 9.82 of the second set is somewhat more intense than it is in the first set.

The spectrums on plate 2 are of the H37Ra fractions. The top spectrum is

that of the single No. 4 fraction produced in the first set of fractionations. The remaining three spectrums are those of the triplicate fractions of the second set made six weeks later. Again all are very much alike, the largest variation being in a greater intensity of the band at 9.79μ of the third spectrum on plate 2.

When the spectrums of H37Rv and H37Ra are compared, it is evident that there are both qualitative and quantitative differences. Qualitatively, the virulent strains have a broad band of medium intensity at 6.40μ; the others do not. Quantitatively, the regions between 7.90 and 9.8μ of the two strains show marked variations in the intensities of the bands, so that the contours of the spectrums of the strains are quite different here.

While fraction No. 4 is very effective in separating H37Rv from H37Ra, it is recognized that another fraction may be found even more effective.

SUMMARY

It has again been demonstrated that it is possible to so standardize the procedures of growth and fractionation of cultures of *M. tuberculosis* that the chemical content of a given fraction is always the same to the degree of producing a characteristic and invariable spectrum.

Also it has been shown that fractions prepared by the same technique from two biologically distinct strains, H37Ra and H37Rv, are sufficiently different chemically that their spectrums can be differentiated.

SUMARIO

Correlación de las Propiedades Biológicas de Cepas del Mycobacterium con sus Espectros Infrarrojos. II. Diferenciación de Dos Cepas, H37Rv y H37Ra, del M. Tuberculosis por Medio de sus Espectros Infrarrojos

Queda demostrado de nuevo que es posible uniformizar a tal punto los procedimientos de crecimiento y de fraccionación que el contenido químico de una fracción sea siempre idéntico y produzca un espectro típico e invariable.

Queda igualmente demostrado que las fracciones preparadas con la misma técnica de dos cepas biológicamente distintas, H37Ra y H37Rv, son suficientemente distintas químicamente para poder diferenciar los espectros de las mismas.

RESUME

Corrélation des propriétés biologiques des souches de Mycobactérium et de leur spectre infra-rouge. II. La différentiation de deux espèces, H37Rv et H37Ra du M. Tuberculosis, au moyen de leur spectre infra-rouge

Il a été de nouveau démontré qu'il est possible de standardiser les procédés de culture et de fractionnement, de telle manière que le contenu chimique d'une fraction donnée est toujours le même, jusqu'au point de produire un spectre caractéristique et non variable.

Il a été également démontré que les fractions préparées par cette même technique à partir de deux souches biologiquement distinctes, H37Ra et H37Rv, ont des caractères chimiques suffisamment différents pour que leur spectre puisse être différencié.

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Editorial

Understanding of Personality Patterns as a Guide for the Rehabilitation of the Tuberculous

It has been said that the best results from the treatment of the tuberculous are obtained when the patient is thought of, not only as possessing diseased organs, but also as a sick personality. It has become increasingly apparent that an adequate understanding of the personality patterns is essential as well as a knowledge of the attendant emotional and psychological maladjustments. Such an understanding is not only essential in the care of the more acute phases of the illness, but also in the rehabilitation period.

A considerable amount of literature has been accumulated on this subject, but there is still much to be learned about it. That tuberculous patients have common personality features has not been generally accepted, but studies have repeatedly revealed that emotional disturbances often precede, and may hasten, the onset of tuberculosis. It is to be clearly understood that by no means is every case of tuberculosis believed to have been precipitated by a disturbed emotional state. The potential of such factors cannot, however, be lightly dismissed. It has been suggested that the advent of the disease is not infrequently the outcome of intolerable mental conflicts and their unsatisfactory solution. In this respect, writers have stressed the function of the illness as a means of flight from frustration and the responsibilities of life, and for self-punishment in the form of organic suicide.

Of the many studies and reports available none has, in the present writer's opinion, so effectively classified the personality types and emotional problems as a publication by Dr. E. Wittkower[1]. The latter has reported on an extensive study of the personalities of 300 interviewed tuberculous patients, which revealed a wide personality diversification. He has attempted to group those individuals with similar basic conflicts and behavior patterns. He classified these groupings as (*1*) the overtly insecure type, which made up 39 per cent of the 300 patients; (*2*) the rebellious types, 12 per cent; (*3*) self-drivers, 27 per cent, self-drivers in reverse, 2 per cent; (*4*) the conflict-harassed type, 13 per cent; (*5*) unclassifiables, 7 per cent. He presents a very instructive description of these various types and their resulting conflicts. There are many other reports in the literature but, to the writer's knowledge, none of these has effectively classified the personality types and emotional problems as they are presented.

A most interesting and, from the writer's point of view, workable plan for the adequate management of the psychological factors and problems has been advanced by Hurst, Coleman, and Hornbein[2]. It is their suggestion that a psychiat-

[1] Wittkower, E.: A Psychiatrist Looks at Tuberculosis, The National Association for Prevention of Tuberculosis, London, 1949.

[2] Hurst, A., Coleman, J. V., and Hornbein, R.: Place of psychiatry in program of tuberculosis hospital, Dis. of Chest, 1949, *15*, 581.

ric service may be oriented to direct work with patients and may be termed "Patient Centered." On the other hand, the psychiatric service may be oriented to the task of helping people who carry the actually day-to-day responsibility for the patient. This latter type of service might be termed a "Staff Centered" psychiatric program. It is believed that a "Patient Centered" psychiatric program in a hospital for the treatment of tuberculous patients tends to become sterile, unproductive, and unrewarding. The attitudes, prejudices, intolerances, and personal problems of the hospital staff are as much a part of the patient's experience with his illness as what goes on in his chest or what goes on in his personality.

A great majority of the psychiatric problems arising in the hospital for the treatment of the tuberculous do not require specialized psychiatric treatment. It is believed by some physicians that, if the attending doctor can deal with the moderate psychic conflicts without bringing a psychiatrist into the picture, the patient's interests are not divided and his confidence is thereby enhanced. If this doctor might have, in accordance with the "Staff Centered" psychiatric program, the opportunity for discussion or consultation with a psychiatrist, it is believed that a greater facility is created in managing patients, together with a better understanding and orientation of the staff. On the other hand, such a "Staff Centered" program does not preclude the direct treatment of the patient by the psychiatrist in the relatively small number of cases in which such a procedure is indicated.

It is misguided and naive to expect a physician not trained in psychiatry to understand the personality structure of the patient, the dynamics of his reaction to illness, medical care, and hospital experience, or the interplay of personality in the staff-patient relationship. Though psychological flair and capacity among physicians and nurses vary greatly, there is little doubt that the opportunity to share in the psychiatrist's thinking and to make use of his time for consultation on cases could be of great value. The role of the psychiatrist in this plan would be that of a participant as a member of the clinical team in the common effort for the general care of the patient.

Consultation would be the basic contribution of the psychiatrist. Such consultation should be available, not only during the initial period of adjustment, but it is equally important that at least the benefit of advice be available throughout the course from hospitalization through rehabilitation and adjustment in the home. A program of education in psychiatric thinking can be carried out in a clinical or hospital group through seminars and group discussions.

It is impossible to separate the early progress of the rehabilitation movement of the tuberculous from that of other diseases. All of the present specialized phases of rehabilitation had their origin in the general problems of the physically handicapped person, and it is only in comparatively recent years that such problems have been given extensive attention. The essential problem of all rehabilitation has to do with mental attitude. The stress should be placed upon the mental ability of the patient to adjust to the handicap as well as upon his physical ability to adjust to the job. One must not forget that it is the individual and not the job which is being rehabilitated. One of the first steps in rehabilitation, therefore, is to awaken the will to recovery and to obtain cooperation and, it might be added, to change "If I get well" to "When I get well."

There are many definitions of the term *rehabilitation*, but we may well consider it as the restoration of the handicapped person to the fullest mental, social, vocational, and economic usefulness of which he is capable. Certain factors are considered vital in attaining a successful rehabilitation. These should include knowing the patient, family understanding, knowing the steps the patient should take to make his economic comeback, knowing the organizations concerned with the patient's adjustment, re-education of the patient, post-sanatorium training, convalescent-stage training, and placement.

The need for guidance in vocational fields and rehabilitation is well recognized as is the need for teaching the patient to recondition himself. The ideal is to establish a rehabilitation department in the sanatorium which should include social work, occupational therapy, and rehabilitation counseling. The social worker is an important element in the team through help in resolving mental and emotional situations, the establishment of a sympathetic bond, and as a contact agent.

The services of a combined rehabilitation-occupational therapy department which can plan and execute diversional and rehabilitation work and direct the interest of the patient into constructive and occupational thinking are needed. During the inactivity enforced by idleness, the patient requires attention and diversion and, when practicable, he should be guided into paths which may lead to future economic independence. This sort of therapy should begin at the bedside and may at first be only diversional, but it tends to make a contented patient. With a low ebb of morale, a discussion of assets is most helpful. Too often in the course of any illness the evaluation of assets is forgotten. To learn to make use of these assets within the setting of one's handicaps and limitations is a major part of getting well and of remaining physically and emotionally adjusted.

Many patients, of course, cannot return to their former occupations and there are some who cannot return to any financially productive fields. For the former, aptitude, interest, and intelligence tests can prove helpful; and for the latter, diversional efforts should be available such as knitting, model making, reading, soap carving, tying flies, or other crafts within the scope of their physical ability.

It is well for the rehabilitation staff to have access to the patient's record, including not only a history of the medical condition but also his prognosis, interests, aptitudes, and basic intelligence. The extent to which this information might be augmented by an understanding of the personality assets and liabilities has already been discussed. There has been some tendency for physicians to assume a too minimal role in the rehabilitation effort. In many instances this may be due to insufficient time, but ideally the rehabilitation conferences should be attended not only by the social worker, occupational therapist, rehabilitation worker, et cetera, but by the physician. This would be an ideal opportunity to obtain the further cooperation of the psychiatrist.

Evidence points to the fact that there is an intimate relationship between the patient's emotional life and what happens to his disease. This is true throughout his course of therapy from diagnosis to occupational return. Assistance in interpretation of the personality problems can, in my opinion, best be made available to the entire team of therapists through a staff-centered psychiatric program.

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Notes

THE ESTIMATION OF ISONICOTINIC ACID HYDRAZIDE (NYDRAZID[1]) IN BIOLOGIC FLUIDS

Isonicotinic acid hydrazide (Nydrazid) has been found to be highly active against experimental tuberculosis in mice (Bernstein, J., Lott, W. A., Steinberg, B. A., and Yale, H. L.; Am. Rev. Tubere., 1952, *65*, 357) and is now undergoing trial in man (Elmendorf, D., Jr., Cawthon, W., Muschenheim, C., and McDermott, W.; Am. Rev. Tubere., 1952, *65*, 429). Hence, it is desirable to have sensitive methods for the estimation of the compound in biologic fluids in order to follow the concentrations attained in the blood, the tissue distribution, and effects on metabolism. The present report describes such a procedure for the estimation of isonicotinic acid hydrazide by extraction and spectrophotometric or colorimetric measurement.

Reagents:

Ammonium sulfate—reagent grade
0.5 *N* sodium hydroxide
Hydrochloric acid: 0.1 *N*, 1 *N*, 6 *N*, 12 *N*
20 per cent isoamyl alcohol-diethyl ether mixture. (The isoamyl alcohol [Merck] should be washed with three equal volumes of 2.5 *N* HCl, followed by an equal volume of 2.5 *N* NaOH and several water washes.)
p-Dimethylaminobenzaldehyde reagent: 1.2 gm. (Eastman Kodak) are dissolved in 20 ml. of absolute ethyl alcohol, and 2 ml. of 12 *N* HCl are added. The reagent should be prepared fresh daily.

Extraction procedure: To 1 to 3 ml. of plasma or urine containing 3 to 50 γ of isonicotinic acid hydrazide (if less than 3 ml. is taken, make up to 3 ml. with distilled water) in a 60 ml. glass-stoppered bottle, add 3.2 gm. of ammonium sulfate, 1 ml. of 0.5 *N* NaOH, and 40 ml. of 20 per cent isoamyl alcohol-ether mixture. For plasma, shake mechanically for thirty minutes and for urine, ten minutes. Centrifuge at 2,500 r.p.m. for five minutes. Transfer 30 ml. of the organic phase to a 60 ml. glass-stoppered bottle containing 4 ml. of 0.1 *N* HCl. Shake for five minutes and centrifuge at 2,500 r.p.m. for approximately three minutes. The acid extract is then separated and taken for analysis. For purposes of standardization, known amounts of isonicotinic acid hydrazide should be added to plasma or urine and treated as above. The amounts recovered are used as standard recovery figures.

Spectrophotometric procedure: The acid extract is read in a Beckman ultraviolet spectrophotometer at 266 mμ in 1 cm. cells, using 0.1 *N* HCl as the blank. The unknown can then be calculated using standard recovery values and concentration versus density data obtained on unextracted solutions of known concentrations. Blanks: (*1*) 0.1 *N* HCl as described; (*2*) an extracted biologic blank read against 0.1 *N* HCl.

Colorimetric procedure: Place 1 to 3 ml. of acid extract containing 1 to 15 γ of isonicotinic acid hydrazide (if less than 3 ml. is taken, make up to 3 ml. with 0.1 *N* HCl) in a 15 ml. graduated conical test tube. Add 0.5 ml. of 6 *N* HCl and 1 ml. of *p*-dimethylaminobenzaldehyde reagent and mix. Heat in a water bath at 100°C. for forty-five minutes. Cool to room temperature, make to 4 ml. with 0.1 *N* HCl (some volume is lost by evaporation), and read in a Beckman spectrophotometer at 450 mμ using 1 cm. cells against a blank of 0.1 *N* HCl. Standards are prepared by taking several concentrations of isonicotinic acid hydrazide in 3 ml. of 0.1 *N* HCl and treating as described. The unknown is then calculated using stand-

[1] Proprietary name, E. R. Squibb & Sons.

ard recovery and concentration versus density data. Blanks: (*1*) 0.1 *N* HCl as described (*2*) extracted biologic blank treated similarly to unknown and read against (*1*).

Discussion: The methods described have been found reliable for the determination of isonicotinic acid hydrazide (Nydrazid) in plasma and urine within the limits mentioned.

Present evidence indicates that the colorimetric test depends upon the reaction of *p*-dimethylaminobenzaldehyde with free hydrazine liberated from isonicotinic acid hydrazide by acid hydrolysis (Pesez, M., and Petit, A.: Bull. Soc. chim. France [Memoirs], 1947, 122). Therefore, free hydrazine could be an appreciable source of error. It has been found, however, that hydrazine reacts immediately with the reagent at room temperature, and the unknown can be so tested. If necessary, a differential analysis can be accomplished by the use of both procedures. No free hydrazine has been detected in the fluids of test animals studied so far. Isonicotinic acid has an absorption peak at 270 mμ in 0.1 *N* HCl and, at concentrations of 50 to 300 γ per 3 ml. of sample, is extracted to the extent of approximately 10 per cent in the system employed. The free acid is negative in the colorimetric method.

Although the application of the methods to biologic specimens indicates satisfactory specificity, other possible sources of error are under investigation.

In summary: Isonicotinic acid hydrazide (Nydrazid) can be extracted from alkalinized plasma or urine into an isoamyl alcohol-ether-ammonium sulfate system and subsequently estimated either spectrophotometrically or colorimetrically. The spectrophotometric method using the absorption peak at 266 mμ is reliable for concentrations of 5 γ per ml. in plasma and 20 γ per ml. in urine. The colorimetric method using *p*-dimethylaminobenzaldehyde as the reagent has a lower limit in plasma of 1 γ per ml. ± 5 per cent, and 5 γ per ml. in urine.

Squibb Institute for Medical Research
New Brunswick, New Jersey
January 19, 1952

Jacques M. Kelly
Raymond B. Poet
(With the technical assistance of
Lois M. Chesner and Antonine Hoffman)

NEW CATHETERIZING BRONCHOSCOPE[1]

The catheterizing bronchoscope provides an accurate method of catheterizing definite bronchi under direct visualization for procuring secretions for laboratory examination and for bronchography. It utilizes the principle of the bronchoscope (Jackson, C., and Jackson, C. L.: Textbook of Disease and Injuries to the Larynx, MacMillan Co., New

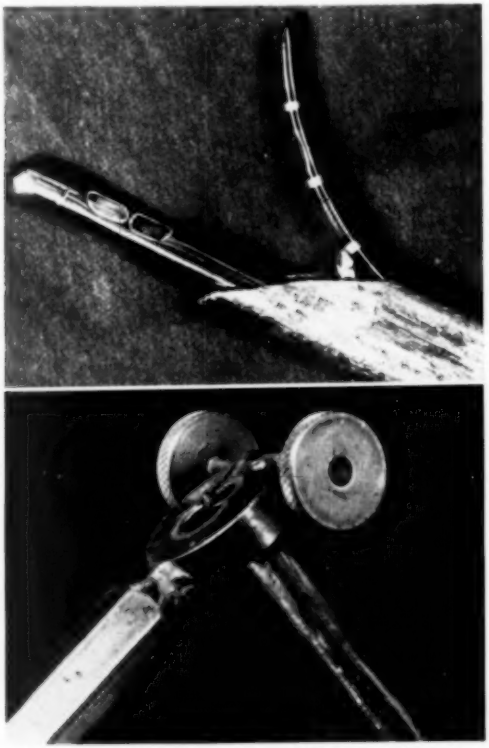


Fig. 1 (Top). Lower end of bronchoscope with right-angle telescope and catheter elevated.

Fig. 2 (Bottom). Upper end of bronchoscope showing knurled knot for controlling deflector.

York, 1942, p. 26) and the catheterizing cystoscope (Vest, S. A.: J. Urol., 1949, *62*, 378) and is similar in principle to the upper lobe bronchoscope and forceps reported by Holinger

[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

and Johnston (Holinger, P. H. and Johnston, K. C.: Tr. Am. Acad. Opth., 1950, March–April, p. 385). It is 8 mm. in diameter and 40 cm. long. It consists of two parallel tubes, the lower one large enough to admit the right-angle telescopic bronchoscope (Broyles, E. N.: Tr. Am. Broncho-Esoph., 1948, 13), and the upper one large enough to admit a No. 8 ureteral catheter or the standard light carrier. At the distal end of the upper compartment there is a deflector controlled by a wheel at the proximal end, which deflects the catheter to the desired angle (figures 1 and 2).

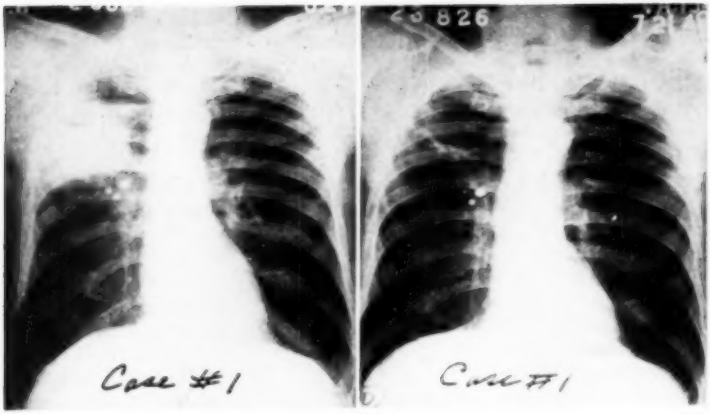
The bronchoscope is introduced in the usual manner, either with or without a laryngoscope, with the light carrier in the upper compartment. The main bronchi and ostia are inspected in the usual manner; the light carrier is then removed and, with the right angle telescope in the larger compartment, a more detailed examination is made of the orifices of the apical, posterior, and anterior branches of the right upper lobe bronchus, the lateral and medial branches of the right middle lobe bronchus, the apical-posterior and anterior branches of the upper division of the left upper lobe bronchus, and the superior and inferior branches of the lower division of the left upper lobe bronchus. Either the fore-oblique telescope or the right-angle telescope is used for all lower lobe bronchi as the position may require.

When an inflamed orifice is found or pus is seen coming from a branch bronchus, a No. 8 ureteral catheter is introduced through the smaller compartment until the end is seen through the telescope. The distance from the main bronchus to the edge of the lung over the bronchus to be catheterized is measured on the postero-anterior film. This measurement is marked on the catheter extending from the proximal end of the scope by attaching a piece of adhesive tape which acts as a stop to prevent the catheter from being inserted too far. When the desired orifice of the bronchus and the tip of the catheter are seen in the telescope, the catheter can be introduced into that bronchus. By using the gentlest pressure, any narrowing or stenosis of the bronchus can be discovered by the resistance offered to the introduction of the catheter. The calibrations on the ureteral catheter, as it extends out of the proximal end of the bronchoscope, indicate the distance of the obstruction from the distal end of the bronchoscope. If this resistance is due to congestion of the membrane, 1 or 2 cc. of neosynephrine instilled through the catheter will shrink the membrane and allow the catheter to pass further. If the obstruction is due to stenosis or a kink, the catheter will not go on and lipiodol may be instilled through the catheter in order to localize the obstruction by roentgenography.

The following three cases illustrate the advantages of the catheterizing bronschoscope:

Case 1. The patient was a 53-year-old man with an acute nontuberculous lung abscess of the right upper lobe. He received treatment with 900,000 units of penicillin for eleven days and 40 gm. of aureomycin daily for three months. At the end of this period, a fluid level could be seen on roentgenograms of the chest. On bronchoscopy the right angle telescope revealed pus coming from the right posterior apical branch. The catheter was introduced into the posterior apical branch but, because of an obstruction, the catheter went only 15 mm. into the bronchus. Neosynephrine instilled through the catheter was followed by a profuse discharge of pus. The catheterization was repeated five times, and the abscess soon healed. The antibiotics may have been chiefly responsible for the healing of the abscess but, since antibiotics are usually of greater benefit in curing an abscess when there is adequate drainage, the use of the catheterizing bronchoscope probably aided and speeded up the healing of the diseased area in this lung (figures 3 and 4).

Case 2. The patient was a 38-year-old male with sputum positive for acid-fast bacilli without roentgenographic evidence of pulmonary disease. Bronchoscopy and the fore-



Case 1

FIG. 3 (Left). Roentgenogram showing abscess density in right mid-lung field.
FIG. 4 (Right). Roentgenogram showing clearing of the abscess density with residual scar.

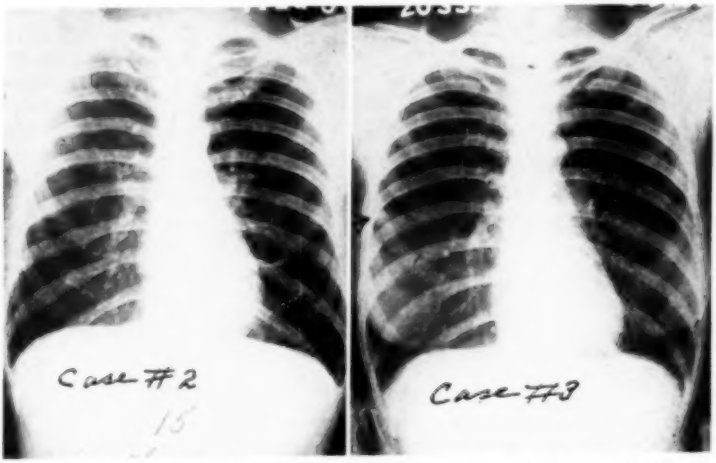


FIG. 5. Case 2 (Left). Roentgenogram showing no evidence of pulmonary tuberculosis but sputum positive for *M. tuberculosis*, found with catheterization of the left posterior basal branch.
FIG. 6. Case 3 (Right). Roentgenogram showing area of density in mid portion of right lung field proved to be tuberculous by culture obtained through catheterization.

oblique telescope revealed inflammation of the left posterior basal branch bronchus. This bronchus was catheterized and washed, and a positive culture for tubercle bacilli was obtained, thus localizing the diseased area (figure 5).

Case 3. The patient was a 50-year-old female whose roentgenograms revealed an area of density in the mid portion of the right lung. The patient's sputum was consistently negative for tubercle bacilli. Bronchoscopy with the right-angle telescope showed congestion of the orifice of the right superior bronchus. Smears and cultures of a swab taken from this orifice were later reported as negative for tubercle bacilli. Following the swabbing, catheterization, and washing of this bronchus, a positive culture for tubercle bacilli was obtained (figure 6).

The catheterizing bronchoscope described above has the following uses:

With the telescope lenses, the bronchoscope affords a means of accurate catheterization of any branch bronchus.

Secretions can be collected nearer their source than in the main stem and beyond the reach of the flexible tip suction.

For bronchograms of a localized area of the lung, lipiodol can be put into a certain desired bronchus under direct vision, and in that way alleviate the necessity of exposing the patient and operator to X-radiation which is necessary when the catheter is placed under fluoroscopic guidance as recommended by Nordenstrom and Norlin (Nordenstrom, B. E. W., and Norlin, U. A. T.: Acta radiol., 1951, *35*, 246).

In some cases, more adequate drainage of lung abscesses can be obtained by placing neosynephrine into the edematous bronchus and allowing for the evacuation of pus.

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SAN FERNANDO, CALIFORNIA
September 11, 1951

Books

THE PUBLIC HEALTH NURSE AND HER PATIENT. Revised edition. By Ruth Gilbert. Pp. 348, published for The Commonwealth Fund, Harvard University Press, Cambridge, Massachusetts, 1951, Price, $3.75.

In the revised and enlarged edition of Miss Gilbert's book, the nursing profession can again take pride in her achievement. Every professional nurse can get needed help in understanding herself and her patients. While the book is written for nurses, it will be of interest to allied professional people who want to learn more about the job of public health nursing.

It will be reviewed here as it relates to nursing the tuberculous patient and his family. However, it must be understood that, even though certain parts of the book are not discussed, such as "Nurse and Maternity Patient" and "The Child in His Family," they are equally important to the nurse and the tuberculous patient and family. The family is the unit of service and tuberculous patients and families have emotional problems relating to pregnancy, and children in these families have "behavior problems." Public health nurses will want to learn more about these problems so they can help the tuberculous patient and family who have them.

The application of the principles and philosophy of mental hygiene gives vitality to nursing. Miss Gilbert states that mental hygiene is not a panacea. It cannot give blanket definitions of the "right" or "wrong" way in working with people. "There is a growing recognition that mental hygiene offers no such short cuts but rather an informed, deliberate, observant method of working, a habit of stopping to think what the behavior of the patient and others may mean in relation to a situation and how the nurse herself relates to it." This statement sets the tone of the book. The selection of case material illustrates the patient's reaction to health and illness situations and the way in which the nurse relates herself, her teaching, and her care to the complex lives of the people she serves.

The establishment of relationship between the nurse and her patient is vividly brought out in the chapter on "Teaching Health." There are those patients who readily accept the nurse and her service because they want her help, and they use it constructively. There are also those patients who accept her and her services because of emotional needs, in which event they may or may not be able to use her help constructively.

Some patients reject the nurse and her services because of deep-seated and complex problems in their lives which make her and her services painful to them. For example, "A young man, formerly a brilliant college student, was a post-sanatorium patient who needed careful follow-up. He told the nurse not to visit him, saying bitterly that he knew all she knew about tuberculosis and did not want to be reminded of his illness by her presence."

The nurse can contribute to resistance on the part of the patient if she does not recognize her own emotional reactions to a given situation. Her judgment may be colored by her own standards which interfere with her ability to stop to think, to try to understand, and to keep from showing disapproval even when she cannot approve of the patient's behavior. It is the non-judgmental, nonauthoritative attitude with patients that helps her to establish the relationship that is basic to her work with all people.

Every public health nurse has had feelings of inadequacy, frustration, and failure

when patients do not respond to her teaching by making the changes in their lives her teachings suggest. Those of us who work with the tuberculous patient and his family can say "Amen" to this passage from Miss Gilbert's book:

> Teaching health frequently must include, for example, the attempt to convince a patient that it is advisable for him to go to a sanatorium or in other ways radically to change the course of his life. Not only is the nurse held responsible for 'progress' in many such instances, but pressure, sometimes political pressure, may be brought to bear on the organization of which she is a member to 'show results.' On the other hand, the nurse is dealing with the complex lives of people. It is a question how far we may intrude on them. Perhaps it is possible to reconcile the two points of view when we realize that our recognition of people's complexity and the ways in which the nurse may relate to her patients and their health problems is just as literally public health nursing as the application of medical facts.

Observing, listening, and responding as purposeful skills which the nurse uses in a variety of ways are ably brought out through case material.

The patient's reaction to his illness, whether acute or chronic, is almost always fear, regression, egocentricity, or preoccupation with himself. The extent to which any of these reactions take hold and dominate the situation and his life will depend upon the kind of a person the patient is and also upon the kind of care and understanding the nurses, doctors, and others give him.

Miss Gilbert says:

> . . . that an understanding of the possible meaning of illness for the patient helps the nurse to evaluate the way he manages his illness as well as her feelings about his behavior as a patient. We may make the mistake of considering the patient who uncomplainingly endures a long illness or recurrence of illness as 'well adjusted' when perhaps he accepts his illness easily because he has an unconscious need for it. He may, in fact, be far more poorly adjusted than the person whose restlessness or rebellion against illness is more troublesome to the nurse.

In discussing the chronically ill patient, this book brings out some of the fears and the anxieties which are particularly acute among tuberculous patients. The tuberculous patient's denial of his illness is most often based on fear. Fear that his whole life will be radically altered; fear of the future, his job; fear that he may have infected others; and fear of hospitalization. Miss Gilbert discusses how nurses can help patients to accept recommended treatment when they understand these fears. In following the recommendation for hospital care, the nurse will not hurry the patient to make a decision to go to the hospital. She will, of course, explain the hospital regimen and the steps necessary to enter the hospital so that he can understand them. She can help him to express his fears by saying that many patients fear going to the hospital because they will be separated from their families for a long time and they do not know what to expect from the treatment in the hospital. Because she seems to understand what he is feeling about his illness, the patient may begin to have confidence in the nurse and the course of treatment she is advising.

The tuberculous patient who refuses re-examinations, or who goes from one doctor to another searching for one who will give him a favorable diagnosis, is familiar to all public health nurses. Miss Gilbert says:

> There is no short cut to helping these patients. Certainly mere insistence on examination will often add to the patient's resistance of bringing him to a physician.

> One can, however, recognize that the emotional basis for this resistance is often fear, expressing itself through a variety of behavior on the part of the patient and his family.

There are many examples in this book of the problems of the tuberculous patient and his family. Most of us who read it will remember situations where we had little understanding of the meaning of our patient's behavior and even less about our own. This book will help us—and all others who are concerned with patients and their families.

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NURSING IN PREVENTION AND CONTROL OF TUBERCULOSIS. Revised edition. By H. W Hetherington and Fannie W. Eshleman. Pp. 361, G. P. Putnam's Sons, New York, 1950, Price, $4.50.

This book has proved its worth as a textbook in tuberculosis nursing during the past ten years since its publication in 1941. It is used in basic courses of tuberculosis nursing as well as a reference text by all nurses.

The work is comprehensive, as it covers the wide area of caring for patients with tuberculosis in the clinic, in the home, and in the hospital; together with the responsibility of the nurse in control of the disease. The affirmative style makes it particularly desirable for nurses who are not well acquainted with all of the clinical aspects of the disease. Controversial subjects (and they are many in this area of nursing) do not often appear as such. The organization of the material concerning paragraph headings is improved, enhancing the use of the book as a reference text. Review questions and a generous list of references at the conclusion of every chapter greatly assist students and instructors. One is impressed with the abundant and reliable documentation throughout. In general, the subject is well presented, easily read, and follows modern concepts of nursing. It is unfortunate that the expressions "asylums" and "inmates of asylums" have been retained.

The authors have enriched the book by drawing upon their many years of experience. One is constantly delighted with familiar details known to those who have spent some years in tuberculosis work.

New material has been added to diagnostic procedures, case-finding, nursing in the treatment of tuberculosis, the chest clinic, and measures of control. Newer concepts of rehabilitation are presented. Illustrations and graphs have been changed, which brings them up to date. The advent of chemotherapy in the treatment of tuberculosis is adequately handled separately in a few pages, without over-dramatization. One wonders why much of this could not have been incorporated into the next chapter which discusses the treatment of pulmonary tuberculosis and its complications.

Although prevention of the disease is integrated throughout, the chapters on "Tuberculosis Prevention Among Nurses" and "Prevention of Tuberculosis" are of particular interest to nurses. The details of health teaching, prevention of airborne contagion, the use of protective clothing procedures in prevention of infection with tubercle bacilli, and BCG vaccination have each been elaborated upon considerably. Certainly a review of these chapters will do much to build confidence and overcome fear in the nurse who is inexperienced in tuberculosis nursing.

Throughout the book the nurse is recognized as a teacher and as a teamworker with all who are interested in the control of tuberculosis. Numerous suggestions are given which should be of support to the nurse in these roles.

Two chapters are devoted to family health services, and family case studies guide the nurse in understanding the importance of the family unit, the problems precipitated by

tuberculosis, and her part in assisting families with plans to meet these problems. Outlines are presented to stimulate the public health nurse and the hospital nurse to participate in clinical and ward conferences.

The revision of this textbook will assure its continued use in the well-deserved place it has established in tuberculosis nursing.

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THE HEALING TOUCH. By Harley Williams. Pp. 408, Charles C Thomas, Springfield, Illinois, 1951, Price, $6.75.

The author of this book is well known to tuberculosis workers throughout the world as the Secretary-General of the British National Association for the Prevention of Tuberculosis. It is perhaps not so well known that he is a barrister-at-law, a novelist, and a medical historian. *The Healing Touch* is the latest of a series of fascinating medical biographies from his hand. It is well printed, in a pleasing format. Although it deals but slightly with the subject of tuberculosis, all readers in that field will find it deeply rewarding for its attractive literary style, its wealth of unusual biographical detail, and above all for its penetrating insight into the personal driving forces of a group of eminent men and women who have inspired medical progress.

ESMOND R. LONG
Philadelphia, Pennsylvania

BOOKS RECEIVED

THE PATHOGENESIS OF TUBERCULOSIS. Second Edition. By Arnold R. Rich. Pp. 1018, with illustrations, Charles C Thomas, Springfield, Illinois, 1952. Price, $15.00

PENICILLIN DECADE, 1941–1951, Sensitizations and Toxicities. By Lawrence Weld Smith and Ann Dolan Walker. Pp. 122, The Arundel Press, Inc., Washington, D. C., 1951. Price, $2.50

LA TUBERCULOSIS EXPERIMENTAL EN EL COBAYO. ACCION DE LA DIHIDROESTREPTOMICINA EN LA TUBERCULOSIS EXPERIMENTAL. Tesis de Doctorado. By Ramon Vargas Machuca. Pp. 78, Editora Medica Peruana S. A., Lima, 1951.

LE DÉPISTAGE RADIOLOGIQUE SYSTÉMATIQUE DES AFFECTIONS DU THORAX (Son Principe—Ses Modalitiés—Son Rendement). By Maurice Bariéty and Charles Coury. Pp. 216, Masson & Cie, Paris, 1952.

VORLESUNGEN ÜBER DIE TUBERKULOSE DES KINDES UND JUGENDLICHEN. By Werner Catel. Pp. 207 with illustrations, Georg Thieme Verlag, Stuttgart, 1951, Price, $3.80.

VERSLAGEN DER TUBERCULOSE-STUDIE-COMMISSIE VAN DE NEDERLANDSE CENTRALE VERENIGING TOT BESTRIJDING DER TUBERCULOSE. Pp. 98, 's-Gravenhage, Riouwstratt, Holland, 1951.

PUBLICACIONES DEL INSTITUTO ANTITUBERCULOSO "FRANCISCO MORAGAS" DE LA CAJA DE PENSIONES PARA LA VEJEZ Y DE AHORROS. Volumen IX. Pp. 143, Instituto Antituberculoso "Francisco Moragas", Barcelona, 1951.

EINFUHRUNG IN DIE RONTGENOLOGIE. By G. F. Haenisch, H. Holthusen, and A. Liechti. Pp. 521, with illustrations, Georg Thieme Verlag, Stuttgart, 1951.

THE COST OF SICKNESS AND THE PRICE OF HEALTH. By C. E. A. Winslow. Pp. 106, World Health Organization, Monograph Series No. 7, World Health Organization, Palais de Nations, Geneva, 1951, Price, $1.50.

SOMETHING CAN BE DONE ABOUT CHRONIC ILLNESS. By Herbert Yahraes. Pp. 32, Public Affairs Pamphlet No. 176, New York, 1951, Price $0.25.

History of Diagnostic Standards and Classification of Tuberculosis of the National Tuberculosis Association

The publication of the 1950 edition of *Diagnostic Standards and Classification of Tuberculosis* by the National Tuberculosis Association brought numerous inquiries concerning the history of its various printings. For its information in answering such queries, the Council of the American Trudeau Society was furnished with a brief outline of the successive issues. The Council felt that the present was an appropriate time to review the origins and course of development of this now highly influential document and requested some expansion of the outline and a wider distribution. The following pages are the result; in them is given an account of the organizational and committee structure concerned with the preparation of the series of editions, and the principal changes in text and new material introduced with the passage of years and growth of understanding of tuberculosis.

Less than a year after the National Association for the Study and Prevention of Tuberculosis was organized in 1904, a letter from the first president, Dr. Edward Livingston Trudeau, was presented at a meeting of the Board of Directors (April 14, 1905) suggesting that a committee of five sanatorium physicians be appointed "to consider the classification of cases of pulmonary tuberculosis." The Board voted that Dr. Trudeau be requested to carry out the intention expressed in his letter, and he thereupon appointed a Committee on Clinical Nomenclature of six members as follows: Dr. Vincent Y. Bowditch, Boston, Chairman; Dr. W. H. Bergtold, Denver; Dr. Lawrason Brown, Saranac Lake, N. Y.; Dr. Herbert Maxon King, Liberty, N. Y.; Dr. George W. Norris, Philadelphia; and Dr. John H. Pryor, Ray Brook, N. Y.

The report of this Committee will be found on page 259 of the TRANSACTIONS of the first annual meeting, held May 18–19, 1905, in Washington, D. C. (1). The Committee refers to Turban's scheme for the method of comparative statistics for pulmonary tuberculosis and quotes from some modifications of this scheme as published in *Tuberculosis*, the monthly publication of the Central International Bureau for the Prevention of Consumption, Berlin. This Bureau was the Executive Office of the International Tuberculosis Congresses, of which the fifth meeting was being held that year in Paris. The Committee then proposed its own classification, and the TRANSACTIONS record the discussions that took place at the meeting. The report of the Committee was approved.

At the 1906 annual meeting (2), the same committee reported again, stating that the previous report had been circulated to more than 100 sanatoriums and that the replies indicated consensus in favor of keeping on with the classification as it then stood. The Committee's opinion was that a further period should be allowed in order that a just estimate of its value might be made.

The American Sanatorium Association, composed of tuberculosis specialists closely associated with the National Association, which had been organized on December 1, 1905, held its third mid-winter meeting on December 14, 1907, and the question of classification was among the topics considered. A committee was appointed consisting of Dr. Lawrason Brown, Chairman; Dr. Harry Lee Barnes; Dr. H. S. Goodall; Dr. Estes Nichols; and Dr. Leopold Rosenberg. This committee was asked to consider the classification

adopted by the National Association, to alter if necessary, and, in any case, to define more clearly and accurately the terminology employed and procedures advocated.

The Committee of the National Association, which had been continued, made a report which will be found on page 144 of the TRANSACTIONS of the 1907 annual meeting (3). Another letter of inquiry had been sent out to sanatoriums and hospitals throughout the United States and Canada, and the results of the answers were included in the report. There was also a discussion of the report, and it was voted to continue the committee.

The Committee on Nomenclature and Classification of the American Sanatorium Association met at the time of the annual meeting of the National Association in Chicago, June 4, 1908, but the attendance was small and the Committee report was laid on the table to be taken up again at the next meeting.

The Committee on Clinical Nomenclature of the National Association made a brief report at the 1908 annual meeting (4), in which reference was made to information contained in the 11th annual report of the Loomis Sanatorium in 1907 and the latest report of the Adirondack Cottage Sanitarium, Saranac Lake. The Committee asked to be discharged.

At the meeting of the American Sanatorium Association in Washington, May 13, 1909, the report of the Committee on Nomenclature and Classification was brought from the table and discussed (5). The chairman was instructed to have the amended report printed in the *Journal of the American Medical Association* and to have reprints provided for each member of the Sanatorium Association (6). The report was printed in the January 30, 1909 (pp. 341-348) issue of the *Journal*.

In the 1912 TRANSACTIONS of the annual meeting (7) will be found the report of the Committee on Nomenclature of the American Sanatorium Association, Dr. Lawrason Brown, Chairman. This report is interesting in that there is a presentation of the question whether the word "cured" should be used, and on this the Committee reported as follows:

> Your Committee believes that the time has now come when it is wise to tell the people, who have thus far been left partly in ignorance, the real facts of the case. They believe that the sanatorium men should take this upon themselves as their duty. They should impart the knowledge that tuberculosis is as curable today and more so than it was ten years ago, but that further study has led us to the knowledge that it is most difficult to say who is cured and when, if such be the case, the cure is arrived at.
>
> For these reasons we believe it wiser to substitute in our terminology the words "apparently arrested" for "apparently cured," and "arrested" for "cured." In place of "arrest" we suggest the use of "quiescent," and in the place of "well," the term "condition of health satisfactory."

This report, having been adopted already by the Sanatorium Association, was referred to the National Association and the Association referred it to the Board of Directors, which voted to appoint a special Committee of Seven "to consider the question of nomenclature, having due regard for international systems of classification, to confer with the American Sanatorium Association on the subject, and to report to the Board of Directors."

The Committee of Seven consisted of the following: Dr. John H. Lowman, Cleveland, Chairman; Dr. E. R. Baldwin, Saranac Lake, N. Y.; Dr. Vincent Y. Bowditch, Boston; Dr. Lawrason Brown, Saranac Lake, N. Y.; Dr. H. R. M. Landis, Philadelphia; Dr. James A. Miller, New York; and Dr. Charles L. Minor, Asheville, N. C. Through meetings and correspondence, the Committee had agreed upon a system of clinical nomenclature, and the report was presented at the meeting of the Clinical Section of the annual

meeting of the National Association on May 8, 1913. After discussion, the report was subsequently approved by the Board of Directors and represented the official system of the National Association. It is printed on pages 243–245 of the TRANSACTIONS (8).

The report distinguished the following stages or types of pulmonary tuberculosis: "incipient, moderately advanced, far advanced, and acute miliary tuberculosis." Definitions for the stages were based principally on functional disturbance. Incipient disease was defined anatomically as "slight infiltration limited to the apex of one or both lungs or a small part of one lobe." The following classification was recommended for designation of clinical status in observations following the initial diagnosis: "apparently cured, arrested, apparently arrested, quiescent, improved, unimproved, and died." "Apparently cured" was defined as that state in which all constitutional symptoms and expectoration of bacilli had been absent for a period of two years under ordinary conditions of life. "Arrested" was defined as that state in which all constitutional symptoms and expectoration of bacilli had not occurred for a period of six months. It was noted in the latter case that the physical signs were to be those of the healed lesion.

In the meantime Dr. Walter L. Rathbun, one of the notable figures in the early work of the National Association, had worked out a rearrangement of the National Association classification at the Municipal Sanatorium of the City of New York, Otisville, New York, in 1910. At the mid-winter meeting of the American Sanatorium Association in New York City, December 11, 1915, under the presidency of Dr. Vincent Y. Bowditch, Dr. Rathbun's scheme of classification was offered for consideration (*See* TRANSACTIONS of National Association, 1916, p. 431). In the first paragraph Dr. Rathbun wrote:

> It is evident from the literature that a satisfactory classification for cases of pulmonary phthisis has been, for a great many years, a most perplexing problem to the men interested in the study of tuberculosis. The present classification of the National Association (TRANSACTIONS, *9*, 1913), while possessing a great many valuable features, is in some ways very unsatisfactory and has been a source of confusion to many men in sanatorium work. I have no intention of suggesting more than a rearrangement of the existing classification.

On motion, the President appointed Dr. Lawrason Brown, Dr. Elliott Washburn, and Dr. Walter L. Rathbun as a committee to report on the matter at the next meeting in May 1916. At the May meeting the Committee presented its report through Dr. Rathbun who had been made Chairman. After a long discussion it was voted to place the matter before the members of the Association to be discussed further at the next meeting.

At that meeting, which was held at Mt. McGregor, N. Y., December 6–7, 1916, the revised classification as proposed was accepted. As Dr. Moorman says in his history of the American Sanatorium Association, "This proved to be an important contribution to the armamentarium of the clinician interested in pulmonary tuberculosis." It should be added that it also proved to be the basic document in the first edition of *Diagnostic Standards*, which was to come out in June 1917. The first number of THE AMERICAN REVIEW OF TUBERCULOSIS, the new scientific publication of the National Association for the Study and Prevention of Tuberculosis, was issued in March 1917 and the first article, entitled "The Classification of Pulmonary Tuberculosis," was written by Dr. Rathbun (9).

In this article will be found a history of previous efforts to arrive at a classification for pulmonary tuberculosis. Dr. Rathbun reviewed the various anatomical and clinical classifications of Bayle, Laennec, Grancher, Fränkel, Brehmer, von Sokolowski, Williams, Loomis, King, Beneke, Cornet, Bard, Koeniger, and Turban. Turban's classification became the basic one in time. The German Imperial Board of Health and the Vienna International Conference on Tuberculosis, in the early years of the twentieth century,

adopted it in modified form. The Turban-Gerhardt classification set up the following categories:

I. Disease of slight severity, limited to small areas of one lobe, which might extend beyond the spines of the scapulae and the clavicles, or, in the case of involvement of one apex only, beyond the second rib anteriorly
II. Disease of slight severity, more extensive than I, but affecting at most the volume of one lobe, or severe disease extending at most to the volume of half a lobe
III. All cases extending beyond the limits of II, and all such with large cavities.

Rathbun noted the fact, however, that in America, as early as 1887, Trudeau had proposed a classification taking into account both physical signs and extent of involvement. He noted also that H. M. King had worked out a classification which was used at the Loomis Sanatorium in 1903. He also mentioned a classification, based principally on Trudeau's scheme, prepared by the American Climatological Association, but no date for this is given.

At the end of the article he printed the classifications as adopted by the National Association for the Study and Prevention of Tuberculosis and the American Sanatorium Association. Pulmonary tuberculosis was divided, previously, into incipient, moderately advanced and far advanced stages, and acute miliary tuberculosis. Dr. Rathbun pointed out that the American Sanatorium Association, at its mid-winter meeting on December 7, 1916, had rearranged the classification on the basis of both lesions and symptoms. The classification adopted, as he pointed out, included incipient, moderately advanced, and far advanced disease, with symptoms rated as A, B, and C.

In 1917 a community demonstration of tuberculosis control was set up in Framingham, Massachusetts. An announcement of a donation of $100,000 from the Metropolitan Life Insurance Company had been made in 1916. It was intended to demonstrate that tuberculosis can be controlled if all the approved resources and methods available are applied. The project was under the direction of a special committee of which Dr. Edward R. Baldwin was Chairman. Dr. Donald B. Armstrong, now Second Vice-President of the Metropolitan Life Insurance Company, was selected as the executive officer of the demonstration. In June 1917 the Community Health and Tuberculosis Demonstration published a pamphlet entitled *Diagnostic Standards in Tuberculosis* (10). The foreword contains reference to the fact that Dr. Baldwin had appointed a special committee known as the Diagnostic Standards Committee and that this committee, working in cooperation with representatives of the demonstration, had prepared this set of standards for diagnosis. It added that the Diagnostic Standards Committee had recommended for use in Framingham the National Tuberculosis Association's classification, with modifications suggested by Dr. Walter L. Rathbun of the Otisville Sanatorium (11).

This, the first formal edition of *Diagnostic Standards in Tuberculosis*, was a more elaborate document than any that had previously appeared. It commenced by defining, for purposes of the classification, loss of weight, loss of strength, fever, elevation of pulse, hemorrhage, family history, exposure, cough, sputum, hoarseness, rales, dullness, altered voice and breath sounds, and D'Espine's sign. It then set up "minimum standards" for the diagnosis of active tuberculosis in childhood, which included history of exposure, the character of constitutional signs and symptoms, the presence or absence of tuberculosis elsewhere, physical signs in the lungs, and the tuberculin test. It was noted that "the X-ray may give valuable confirmatory evidence," but added "a definite diagnosis of tuberculosis is not justified on X-ray examination alone."

The second section of this document dealt with the diagnosis of pulmonary tuberculosis in adults with sputum negative for tubercle bacilli. Similar definitions of symptoms and signs were given, and again a set of minimum standards was set up. This emphasized constitutional factors and required the recognition by technical procedures of some pulmonary abnormality. The variable significance of lesions of the apices and basal portions of the lungs was pointed out, and reference was made to the presumptive relationship of pleurisy with effusion and tuberculosis. This section was followed by the formal classification suggested by Dr. Rathbun and adopted by the American Sanatorium Association at its December 1916 meeting. The pamphlet closed with a two-page statement on standards for treatment of pulmonary tuberculosis in adults, dividing the latter into "suspicious" cases, cases in which the diagnosis was definite, and arrested or apparently arrested cases. It ended with the sound observation that "common sense, optimism, patience, and tact are essential factors in treatment."

A second edition (12) was printed in October 1917 under the direction of the same committee, and only a few minor additions and changes were made. The third edition (13) entitled *Standards for the Diagnosis, Classification and Treatment of Pulmonary and Glandular Tuberculosis in Children and Adults* was published in September 1918. Again, this was under the same committee, but for the first time the conclusions of a committee on tuberculous cervical adenitis were added. These conclusions were outlined in a special section on the diagnosis of tuberculous adenitis, which emphasized the following: enlargement of the cervical lymph nodes, exposure to tuberculosis from human or bovine sources, constitutional signs and symptoms associated with enlarged cervical nodes, the relation of diseased tonsils and carious teeth to enlarged cervical nodes, and the differential diagnosis of tuberculosis in these organs. The section concluded with stress on the importance of pathological examination and confirmatory evidence such as that obtained by the tuberculin skin test. Simple rules were appended for the treatment of tuberculous cervical adenitis, which included tuberculin, surgery, heliotherapy, X-radiation, and hygienic measures.

Except for the latter section, the publication was similar to previous editions. For the record, it should be stated that two Diagnostic Standards Committees served in the preparation of this edition. One of these dealt with pulmonary tuberculosis; it consisted of the following: Dr. Arthur K. Stone, Chairman; Dr. Edwin A. Locke; Dr. Cleaveland Floyd; Dr. John B. Hawes, 2nd; Dr. Elliott Washburn; Dr. Vincent Y. Bowditch; Dr. Eugene R. Kelly; Dr. Henry C. Clapp; Dr. Roger I. Lee; and Dr. Richard Smith. The other Committee was on Tuberculous Cervical Adenitis. Its membership was as follows: Dr. Arthur K. Stone, Chairman; Dr. Frank B. Mallory; Dr. Fritz B. Talbot; Dr. R. A. Coffin; Dr. Channing Simmons; Dr. John B. Hawes, 2nd; Dr. Allen K. Krause; Dr. L. M. S. Miner; and Dr. Charles R. Turner. These committees were continued for the fourth and fifth editions.

The fourth edition (14) was the first one to present *Diagnostic Standards* as a publication of the National Tuberculosis Association, which was the name that in 1918 replaced "The National Association for the Study and Prevention of Tuberculosis." The fourth edition changed the order of the sections on childhood and adult tuberculosis, giving priority to the latter. In both sections strong emphasis was placed on the history of exposure to tuberculosis in childhood. It will be noted that in all of the early editions pulmonary tuberculosis in childhood was given a prominent position. This edition, like previous ones, included the classification of pulmonary tuberculosis as adopted by the National Association for the Study and Prevention of Tuberculosis, with the modifications suggested by Dr. Rathbun. It included also a Functional Tuberculosis Classification

Chart for convenience in keeping records. This edition was issued by the National Association and was published in March 1920. A fifth edition (15), again similar except for minor rearrangements, was issued by the Association in October 1922.

A sixth edition (16) was issued by the Association in April 1926. This was a new presentation embodying the substance of previous editions but with modifications suggested by a new Diagnostic Standards Committee appointed by the President of the NTA in 1925. This Committee consisted of the following: Dr. John A. Smith, Chairman; Dr. Fred H. Heise; Dr. Alexius M. Forster; Dr. Henry D. Chadwick; Dr. Clarence L. Hyde; Dr. Walter L. Rathbun; and Dr. J. Burns Amberson, Jr. In the opening paragraph of the sixth edition five principal criteria for diagnosis of pulmonary tuberculosis in both adults and children were set forth. These were as follows: (*1*) history of hemoptysis of one dram or more without any other known cause, (*2*) history of an otherwise unexplained pleurisy with effusion, (*3*) definite rales which persisted for a week or more in the upper half of the chest, (*4*) definite evidence of parenchymal changes in roentgenographic films, usually in the upper half of the chest, and (*5*) demonstration of tubercle bacilli in the sputum on two or more occasions. Following the definitions of symptoms, which were substantially as given in previous editions, a small and most important section on "examinations" was inserted, the opening paragraph of which stated significantly "It is now recognized that the proper taking of X-rays, preferably stereoscopic, is an essential procedure in examination of the chest." From this time forward, as study of the succeeding editions shows, less and less emphasis was placed on physical examination and more and more on the appearance of abnormalities in roentgenograms.

The sixth edition also carried a statement on methods of determination of clinical activity. This statement stressed the need for correlation of clinical, laboratory, and roentgenographic data during the period of observation, preferably in a sanatorium. It called attention to the fact that progressive disease may exist without the presence of symptoms, and emphasized the desirability of repeated physical examination and serial roentgenograms in determining activity. Significantly, also, in light of the final conclusion of the committee in charge of the edition which appeared in 1950, it stated that "The appearance of tubercle bacilli in the sputum does not in itself mean activity of disease, but does indicate a less favorable type of lesion."

Another significant change was the combination of sections, previously separated, into one section entitled "The Diagnosis of Pulmonary Tuberculosis in Adults and Children." A subsequent section dealt with the diagnosis of hilum tuberculosis; this noted the fact that hilum tuberculosis is usually found in children, but may occur in adults, particularly in Negroes. This section listed "prominent trunks with definite beaded appearance or nodular in outline, extending from the hilum" as one of the characteristic roentgenographic findings of hilum tuberculosis, a pronouncement that probably led to much over-diagnosis in succeeding years. This edition, which represented the most extensive change from previous editions thus far, classified tuberculosis for the first time as minimal, moderately advanced, and far advanced (with the word "incipient" in quotes after minimal). The anatomical and symptomatic classification was essentially like that followed in previous years, but a more extensive description of the physical and roentgenographic findings in minimal, moderately advanced, and far advanced disease than had ever appeared before was given.

The seventh edition (17) was issued in November 1926 under the auspices of the same committee. It is a reprint of the previous edition.

Another reprint appeared as the eighth edition (18) in May 1928. This was brought out by the same committee which reprinted the previous edition.

In January 1931 the Association issued a ninth edition (19) under the auspices of a new committee appointed by the President of the Association in 1929. This committee consisted of the following: Dr. Fred H. Heise, Chairman; Dr. J. Burns Amberson, Jr.; Dr. Henry D. Chadwick; Dr. Stephen A. Douglass; Dr. Cleaveland Floyd; Dr. Clarence L. Hyde; Dr. F. Maurice McPhedran; and Dr. H. E. Kleinschmidt, Secretary. This edition contained a new presentation of *Diagnostic Standards* as prepared by the Committee. The 1931 edition also departed extensively from the editions previously published. It incorporated a new section on "Technical Procedures" in which an outline was given of methods for carrying out the tuberculin test, roentgenographic examination, and procedures for the detection of tubercle bacilli in sputum and other body fluids. The intracutaneous tuberculin test was designated as the procedure of choice. The description of roentgenographic technique was lengthy and detailed. In this edition an attempt was made to correlate the clinical features of tuberculosis more accurately than ever before with the pathological processes concerned. The opening chapter on the "Sequence of Events Following Infection" signalized the new trend. The material presented in that chapter was entirely new; it represented the culmination of a long period of research on the relationship of allergy and immunity and the distinction between primary and reinfection types of disease in tuberculosis in children and adults. The second section of the pamphlet outlined precisely the characteristic differences between childhood and adult types of tuberculosis.

The distinctions listed are those considered as holding today between primary forms in children and reinfection forms in adults. Then followed chapters on the diagnosis and classification of the childhood and adult types of tuberculosis. The stand was taken that the reinfection of adults might be endogenous or exogenous. This edition introduced the present definitive limits of minimal tuberculosis in terms of the second chondrosternal junction and fourth and fifth vertebral spines. The previously accepted classification of cases on the basis of severity of symptoms was abandoned in this edition. The 1931 edition proposed a descriptive classification of lesions as observed in roentgenograms in which the terms "exudative" and "productive" were employed, which, as is well known, proved to be a source of contention in later years.

In 1935 the same Committee wrote a revision which was published as the tenth edition (20). This edition introduced many changes in arrangement. The classification of childhood-type tuberculosis was simplified. It included (*1*) parenchymal lesions, (*2*) tracheobronchial lymph node lesions, and (*3*) combined parenchymal and tracheobronchial lymph node lesions. This edition recommended PPD tuberculin as the reagent of choice in the Mantoux test. The terminology for classification of patients with adult-type tuberculosis was the same as that given in previous editions. Recommendation for a qualitative classification employing the terms "exudative" and "productive" was still carried but with conspicuous reservation in view of the practical difficulties which had become apparent.

The President of the NTA in 1936 appointed another committee, and Dr. Fred H. Heise again served as Chairman and Dr. H. E. Kleinschmidt as Secretary. Other members of the Committee were: Dr. J. Burns Amberson, Jr.; Dr. G. Burton Gilbert; Dr. R. H. Kanable; Dr. John H. Korns; Dr. F. M. McPhedran; Dr. Max Pinner; Dr. C. A. Stewart; and Dr. P. A. Yoder. The Committee issued a tentative edition (21) in 1938 and in the foreword stated that, shortly after the publication of the tenth edition, questions were raised by various clinicians and pathologists concerning certain classifications and interpretations of terms. In appointing a new committee, the President had tried to select members who would take into consideration the varying viewpoints and seek to work

out composite statements which would be acceptable to all, or at least to the majority. The manuscript prepared by the Committee had been sent to a number of clinicians in order that the new classification might be tried out in actual practice. It was clearly evident that, since publication of the 1935 edition, objection had arisen to the terms childhood-type and adult-type tuberculosis. These were replaced in the 1938 edition by "primary tuberculosis" and "reinfection tuberculosis." The volume opened with a section on "Pathogenetic Development of Pulmonary Tuberculosis," which was a manifest attempt to state principles on the relationship of primary and reinfection tuberculosis with which the majority of tuberculosis specialists agreed. As the introduction stated, the classification preserved the old features of extent of lesions and symptomatology, but included others to aid in identifying the pathogenesis of the lesion and to assist in determining its prognostic significance. The second section attempted to correlate the clinical course of tuberculosis with current pathological conceptions. The edition included a chart for convenience in classifying and summarizing chest lesions in individual cases. A definite growth in knowledge of tuberculous lesions from all points of view is evident in this now much enlarged edition. The terms employed to designate the character of the disease as observed after its diagnosis were "quiescent," "apparently arrested," "arrested," "apparently cured," and "unstable (or active)."

In the 1938 edition, for the first time a section was introduced on "Tuberculosis Control." This recommended a nationwide program for detecting active and potential human sources of contagion as an ideal, and stated that such a program required repeated application of tuberculin tests and periodic clinical roentgenographic and laboratory examinations of each infected person.

The definitive edition (22) of *Diagnostic Standards* was issued as a 1940 edition by the Association. Within the two-year period between the tentative edition of 1938 and the definitive edition of 1940, dissatisfaction had again developed over terminology with respect to primary and reinfection-type tuberculosis. In the definitive 1940 edition, primary and reinfection "phases" were discussed. Otherwise the section on pathogenetic development of pulmonary tuberculosis was essentially the same in the two editions. The section on "Diagnosis of Tuberculosis" divided the pulmonary disease into pulmonary tuberculosis with and without symptoms. Students of the long series of editions will note a continuing removal of emphasis in the later editions on the subject of primary tuberculosis in children, reflecting a lessening prevalence of the disease in the American population.

The definitions of pulmonary lesions in terms of extent remained the same as in the preceding edition. Under clinical status, the 1940 edition substituted "(frankly) active" for the previously "unstable (active)."

In preparation for the latest edition (1950) of *Diagnostic Standards and Classification of Tuberculosis* the President of the American Trudeau Society, which is the medical section of the National Tuberculosis Association, was authorized in December 1944 to appoint a Committee on Revision of Diagnostic Standards. The Committee began work in 1945. The membership of the Committee was changed slightly during its six years of work; at the time of publication it consisted of the following persons:

Ralph Horton, M.D., Chairman
Howard W. Bosworth, M.D.
Ezra Bridge, M.D.
W. Edward Chamberlain, M.D.
Herman E. Hilleboe, M.D.
Edgar M. Medlar, M.D.
Oscar A. Sander, M.D.
John D. Steele, M.D.
George J. Wherrett, M.D.
Roy A. Wolford, M.D.

There were four subcommittees, with membership as follows:

Subcommittee on Evaluation of Laboratory Procedures:

C. Eugene Woodruff, M.D., Chairman
Emil Bogen, M.D.
Edwin A. Doane, M.D.
M. L. Furcolow, M.D.
Max B. Lurie, M.D.
Edgar M. Medlar, M.D.
William Steenken, Jr.

Subcommittee on Tuberculin Testing:

Joseph D. Aronson, M.D., Chairman
Florence B. Seibert, Ph.D.
Carroll E. Palmer, M.D.
Sol Roy Rosenthal, M.D.

Subcommittee on Clinical Classification:

John D. Steele, M.D., Chairman
Robert J. Anderson, M.D.
Howard W. Bosworth, M.D.
Ralph Horton, M.D.
Carl Muschenheim, M.D.
J. N. Wilson, M.D.

Subcommittee on Pathology:

Edgar M. Medlar, M.D., Chairman
Morton E. McCutcheon, M.D.
H. Stuart Willis, M.D.
C. Eugene Woodruff, M.D.

This edition (23) is referred to in the introduction as the ninth edition. In preparing the pamphlet for publication, the Publications Unit of the National Tuberculosis Association made an examination of all previous editions and discovered that the Association had incorrectly labeled some as new editions which were merely reprintings with very few changes. A correct listing was then made of those printings which could be justifiably labeled as new editions on the basis of appointment of a new committee and change in text or incorporation of new material. Therefore, the 1950 edition is now referred to as the ninth edition.

The 1950 edition of *Diagnostic Standards* includes a revision of previous material on the pathogenesis of tuberculosis, the clinical course of the disease as correlated with pathological concepts, and the diagnosis of tuberculosis by roentgenographic and laboratory procedures. New material was added on a screening classification for mass roentgenographic surveys, which were in their infancy at the time of the 1940 edition but which had become a most important part of tuberculosis control programs in the interim. In the interest of precision in diagnosis, both text material and a chart were added on the bronchopulmonary segments of the lung. The volume ended with a short chapter on rehabilitation.

The 1950 edition reflects a definite dissatisfaction with the sharp distinction previously made between primary and reinfection tuberculosis. Emphasis is laid on the fact that a large proportion of the tuberculosis seen in adults as well as in children in the present decade is of primary rather than of reinfection character. The term "progressive tuberculosis" is used without reference to its primary or reinfection origin.

Much improvement was made in the description of technical procedures. New roentgenographic equipment had been developed. Miniature film screening techniques were now widely employed, and a screening classification was introduced. The new technique of planigraphy had been devised, and its use was indicated.

The new edition is realistic in its approach to the great problem of activity of tuberculous lesions in the light of new advances in laboratory diagnosis. The classification in the 1950 edition authorized the designation of lesions as "arrested" if they satisfy the

usual clinical and roentgenographic requirements even though tubercle bacilli are occasionally demonstrated in the sputum by refined modern methods. At the same time the classification was considerably simplified and thereby made more practical for reporting cases. "Inactive" replaced "apparently cured"; "arrested" covered the cases previously categorized as "arrested" and "apparently arrested"; the simple term "active" included both of the previous groups designated "quiescent" and "(frankly) active." A final category was included for temporary coding purposes, viz., "activity undetermined." Because of the wide use of the terminology of the 1940 edition in official documents, the 1950 edition carried the two classifications in parallel columns to permit easy translation of the old into new terms.

Diagnostic Standards and Clinical Classification of Tuberculosis is now widely used in many countries as an authoritative document. For local use it has been translated into other languages. However, it is important to note that the committees concerned with the preparation of its successive editions have never looked upon the "standards" or "classifications" as static. With the passage of years since the first edition in 1917, much has been learned about the nature of tuberculosis. Although the purpose of all editions has been the same, and although a strong cord of continuity runs through the entire series, the last edition represents a considerable departure in text material from the first. The most recent committee emphasized the ephemeral nature of standards, and brought out the 1950 edition conservatively as a statement of principles rather than a set of rules, in the hope that it would serve as a good general guide to be employed in practice with such modifications as seemed appropriate in individual circumstances.

ESMOND R. LONG
FREDERICK D. HOPKINS

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NOTES

HISTORY OF DIAGNOSTIC STANDARDS

Attention is called to the "History of *Diagnostic Standards*" on page 494 in this issue of the REVIEW. The office of the American Trudeau Society is anxious to obtain several complete sets of the various editions and printings of *Diagnostic Standards*. Members of the Society who have in their files copies of editions prior to that of 1935 and are willing to give them to the Society are requested to send them to the Executive Secretary of the American Trudeau Society, 1790 Broadway, New York 19, N. Y.

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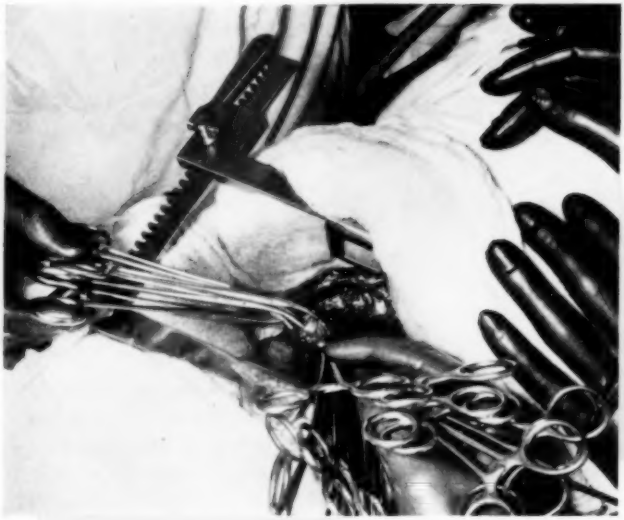

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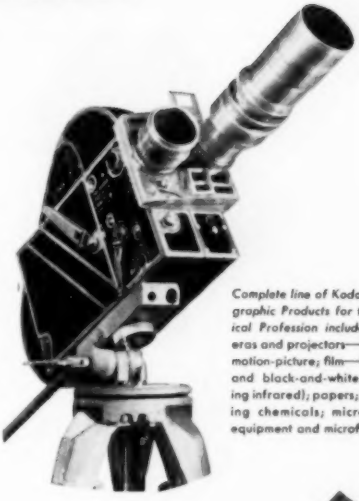

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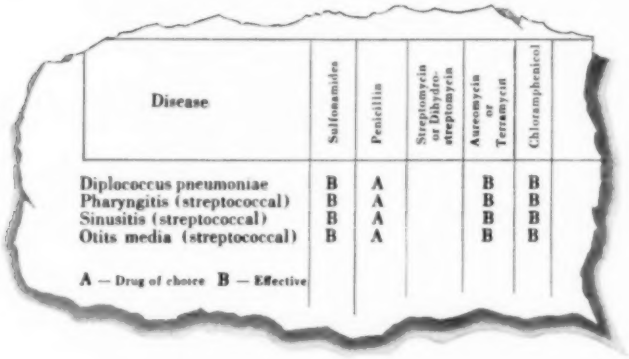

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NATIONAL TUBERCULOSIS ASSOCIATION

ANNUAL MEETING ***MAY 26–29, 1952***

BOSTON, MASSACHUSETTS

The annual meeting of the National Tuberculosis Association will be held in Boston, Massachusetts, May 26–29, 1952. The Statler Hotel will be headquarters.

Some 1500 rooms have been promised by the hotels listed below for the accommodation of those attending the meeting. Arrange to share a twin bed room if possible.

Application for room reservations should be made directly with the hotel of your choice.

Hotel	*Address*	*Single*	*Twin*
Bellevue	21 Beacon Street	$4.50– $7.00	$9.00– $13.00
Bradford	275 Tremont Street	$5.25– $7.50	$8.80– $11.00
Copley Plaza	Copley Square	$5.84– $7.85	$9.90– $14.00
Copley Square	Copley Square	$4.00	$7.00– $8.80
Kenmore	Kenmore Square	$5.50– $8.50	$9.50– $12.50
Lenox	Exeter Street	$4.50– $5.50	$9.00
Manger	North Station	$4.75– $7.75	$8.00– $10.50
Parker House	School Street	$6.00– $8.00	$10.00–$14.00
Pioneer	410 Stuart Street	$3.50– $3.75	$5.50– $7.00
Shelton	91 Bay State Road	$5.00– $7.00	$8.00– $10.00
Somerset	400 Commonwealth Ave.	$7.00– $8.00	$12.00
Statler	Park Square	$6.00–$10.00	$12.00–$16.00
Touraine	62 Boylston Street	$5.00– $7.00	$9.00– $12.00
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University Club	40 Trinity Place	$6.00	$8.00

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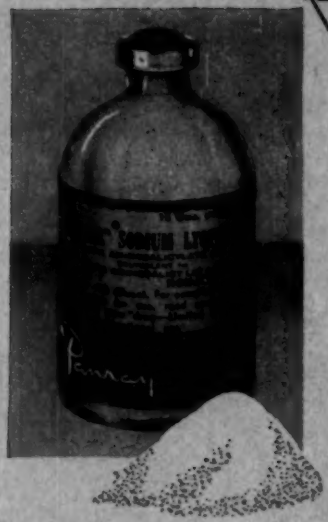

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REFERENCES: 1. "Current Status of Antimicrobial Therapy," Amer. Rev. Tuberculosis, 1951, 63, 573. 2. [illegible] W., Extrapleural Pneumothorax in Collapse Therapy," J. Thoracic Surg., 1951, 21, 217. 3."Meningeal and Acute Miliary Tuberculosis," Trans. 10th VA Conference Chemotherapy of Tuberculosis, January 1951, pp. 360-369.

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